PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 19 September 2000 (19.09.00)	
International application No. PCT/US99/26671	**Applicant's or agent's file reference** 6750-018-228
International filing date (day/month/year) 12 November 1999 (12.11.99)	**Priority date** (day/month/year) 13 November 1998 (13.11.98)
Applicant BURCH, Ronald, M. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

12 June 2000 (12.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Diana Nissen
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

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NOTIFICATION CONCERNING
AMENDMENTS OF THE CLAIMS

(PCT Rule 62 and
Administrative Instructions, Section 417)

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
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PCT/US99/26671	12 November 1999 (12.11.99)
Applicant	
EURO-CELTIQUE, S.A. et al	

The International Bureau hereby informs the International Preliminary Examining Authority that no amendments under Article 19 have been received by the International Bureau (Administrative Instructions, Section 417).

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Huber & Schüßler
Patentanwälte
16. NOV. 2002
Frist: .....

Datum/Date
15.11.02

Zeichen/Ref./Réf.	Anmeldung Nr./Application No./Demande n°./Patent Nr./Patent No./Brevet n°.
E 2047EU	99960285.7-2402-US9926671

Anmelder/Applicant/Demandeur/Patentinhaber/Proprietor/Titulaire
Euro-Celtique, S.A.

COMMUNICATION

The European Patent Office herewith transmits as an enclosure the European search report for the above-mentioned European patent application.

If applicable, copies of the documents cited in the European search report are attached.

[X] Additional set(s) of copies of the documents cited in the European search report is (are) enclosed as well.

EUROPÄISCHES PATENTAMT - EUROPEAN PATENT OFFICE - OFFICE EUROPÉEN DES BREVETS
DEN HAAG - THE HAGUE - LA HAYE

REFUND OF THE SEARCH FEE

If applicable under Article 10 Rules relating to fees, a separate communication from the Receiving Section on the refund of the search fee will be sent later.

European Patent Office

SUPPLEMENTARY PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 99 96 0285

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Y	DIEKMAN ALAN B ET AL: "Sperm antigens and their use in the development of an immunocontraceptive." AMERICAN JOURNAL OF REPRODUCTIVE IMMUNOLOGY, vol. 37, no. 1, 1997, pages 111-117, XP002049805 ISSN: 1046-7408 * the whole document * ---	1-31	C07K16/00 //C07K14/47, C07K16/00
P,Y	WO 99 25378 A (EURO CELTIQUE SA) 27 May 1999 (1999-05-27) * page 5, line 14 - line 17 * * page 16, line 31 - page 17, line 16 * * page 6, line 1 - line 11 * * page 19, line 25 - page 20, line 21 * * claims 63-66 * --- -/--	1-31	

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

A61K
C07K

The supplementary search report has been based on the last set of claims valid and available at the start of the search.

INCOMPLETE SEARCH

The Search Division considers that the present application, or some or all of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for the following claims:

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

Although claims 21-31 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Place of search	Date of completion of the search	Examiner
MUNICH	28 October 2002	Irion, A

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C20)

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	US 5 508 386 A (SOLLAZZO MAURIZIO ET AL) 16 April 1996 (1996-04-16) * column 5, line 64 - column 6, line 26 * * column 10, line 49 - column 11, line 19 *	1-31	
A	PROBA KARL ET AL: "A natural antibody missing a cysteine in V-H: Consequences for thermodynamic stability and folding." JOURNAL OF MOLECULAR BIOLOGY, vol. 265, no. 2, 1997, pages 161-172, XP002217911 ISSN: 0022-2836 * page 161, left-hand column, paragraph 1 - right-hand column, paragraph 1 * * page 162, left-hand column, paragraph 2 *	1-31	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
A	LANGEDIJK ANNETTE C ET AL: "The nature of antibody heavy chain residue H6 strongly influences the stability of a VH domain lacking the disulfide bridge." JOURNAL OF MOLECULAR BIOLOGY, vol. 283, no. 1, 16 October 1998 (1998-10-16), pages 95-110, XP000944533 ISSN: 0022-2836 * abstract * * page 102, right-hand column, paragraph 3 - paragraph 5 * * page 96, left-hand column, paragraph 2 - paragraph 4 *	1-31	

-/--

2

EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 99 96 0285

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	RUDIKOFF S ET AL: "Functional antibody lacking a variable-region disulfide bridge" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 83, October 1986 (1986-10), pages 7875-7878, XP002142019 ISSN: 0027-8424 * abstract * ---	1-31	
A	ZAGHOUANI H ET AL: "Presentation of a viral T cell epitope expressed in the CDR3 region of a self immunoglobulin molecule" SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, US, vol. 259, no. 5092, 8 January 1993 (1993-01-08), pages 224-227, XP002177700 ISSN: 0036-8075 * abstract * ---	1-31	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
A	BRUMEANU T-D ET AL: "Engineering of doubly antigenized immunoglobulins expressing T and B viral epitopes" IMMUNOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS BV, NL, vol. 2, no. 2, 1 June 1996 (1996-06-01), pages 85-95, XP004052673 ISSN: 1380-2933 * abstract * * page 94, right-hand column, paragraph 2 - page 95, left-hand column, paragraph 2 * * page 86, right-hand column, paragraph 2 * -----	1-31	

2

EPO FORM 1503 03.82 (P04C10)

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 99 96 0285

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

28-10-2002

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9925378 A	27-05-1999	AU 1459799 A	07-06-1999
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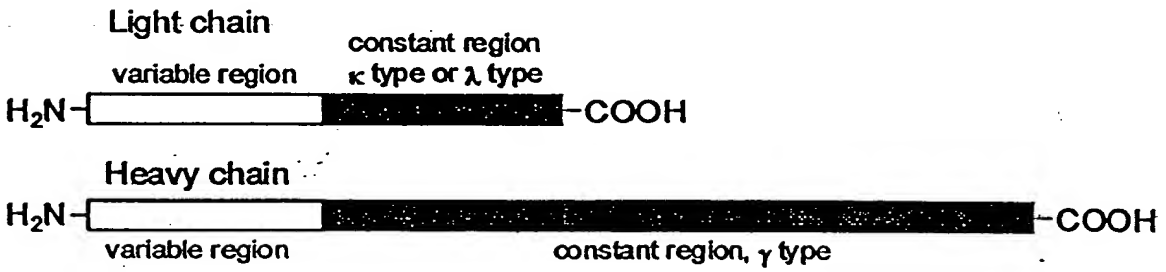

Light chain
variable region
constant region
κ type or λ type
H2N
COOH
Heavy chain
H2N
COOH
variable region
constant region, γ type

FIG. 1

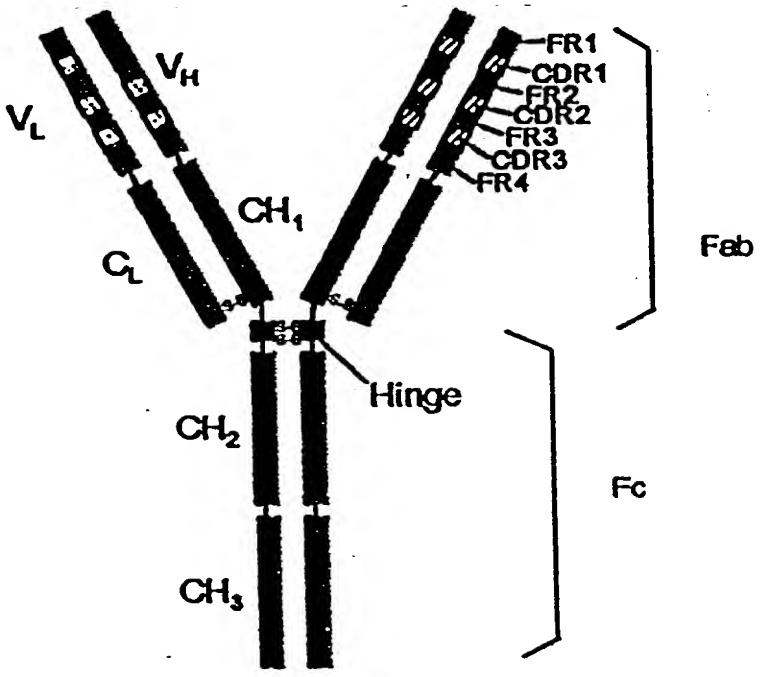


FIG. 2

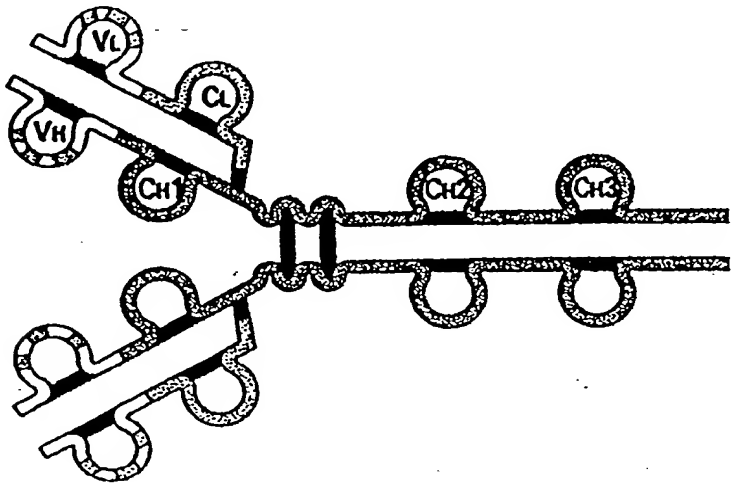


FIG. 3

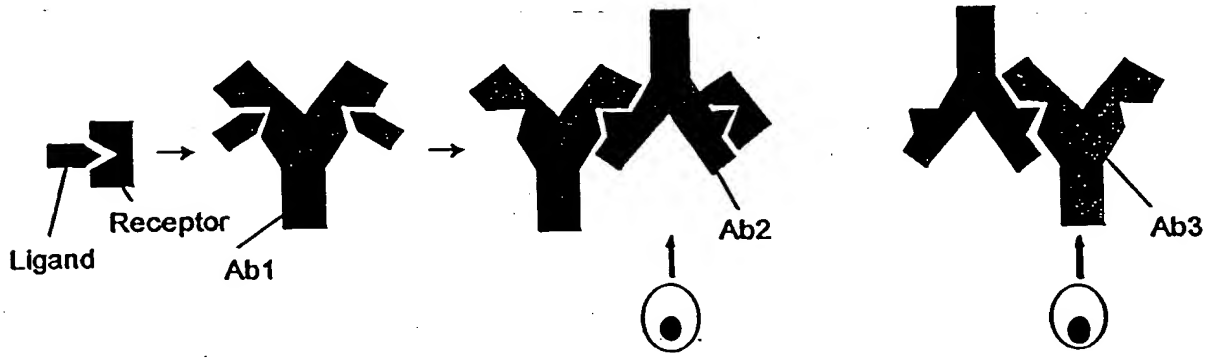


FIG. 4

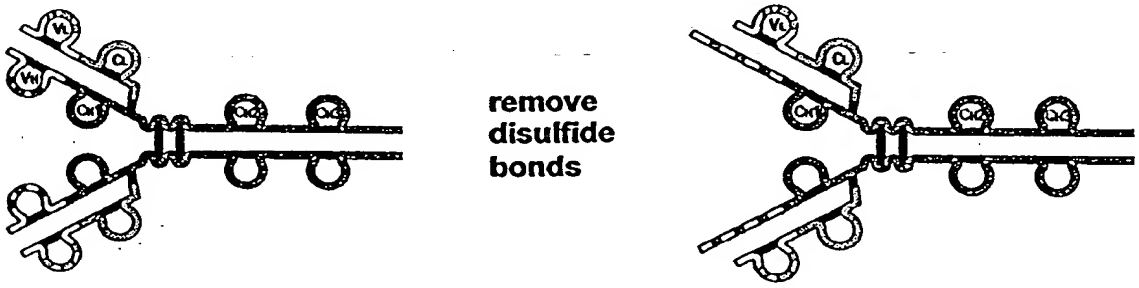


FIG. 5

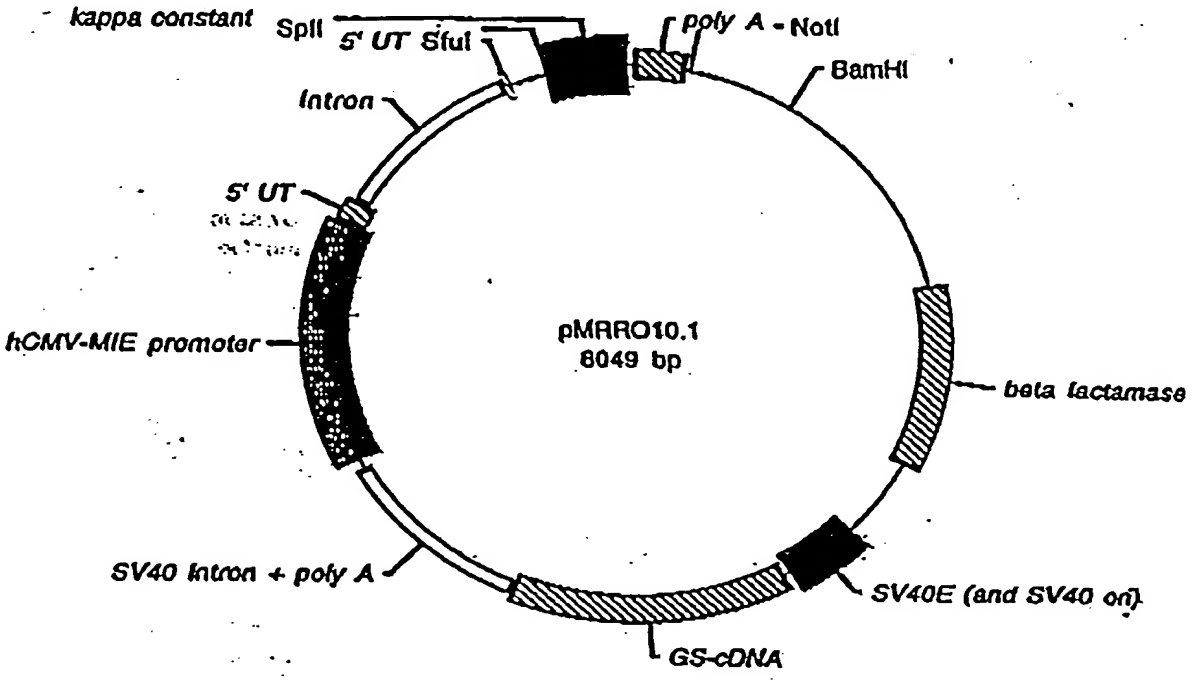

kappa constant
SpII
5' UT SfuI
poly A - NotI
BamHI
Intron
5' UT
hCMV-MIE promoter
pMRRO10.1
8049 bp
beta lactamase
SV40 Intron + poly A
SV40E (and SV40 ori)
GS-cDNA

FIG. 6A

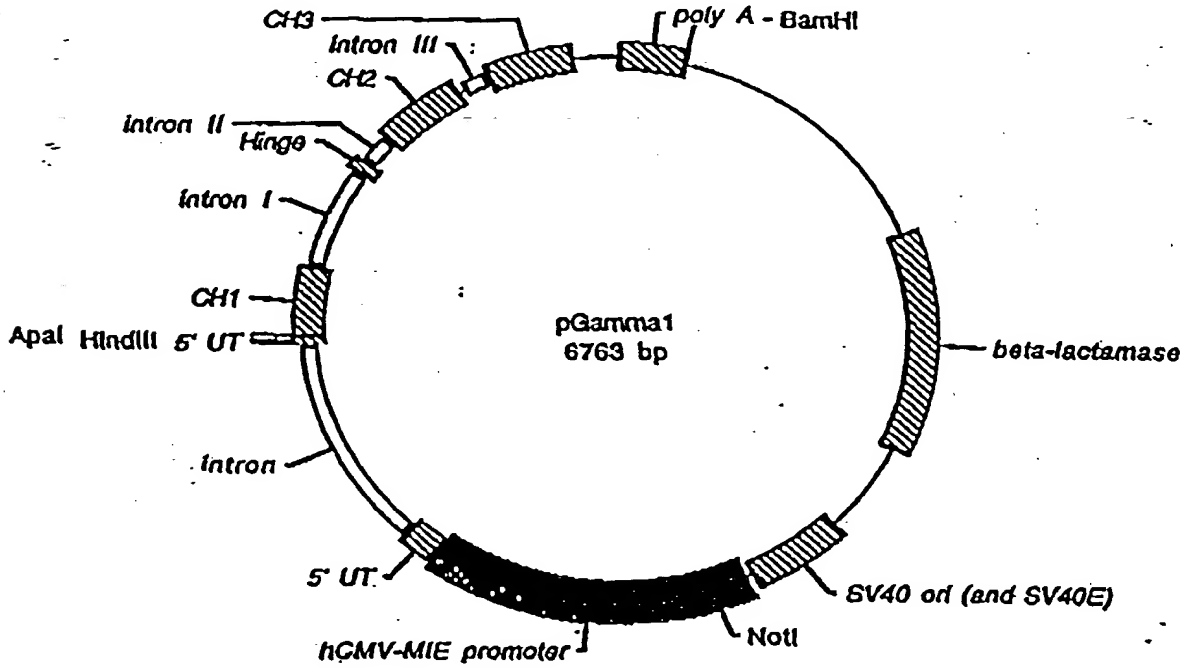

CH3
Intron III
CH2
Intron II
Hinge
Intron I
CH1
ApaI HindIII 5' UT
Intron
5' UT
hCMV-MIE promoter
NotI
SV40 ori (and SV40E)
beta-lactamase
poly A - BamHI
pGamma1
6763 bp

FIG. 6B

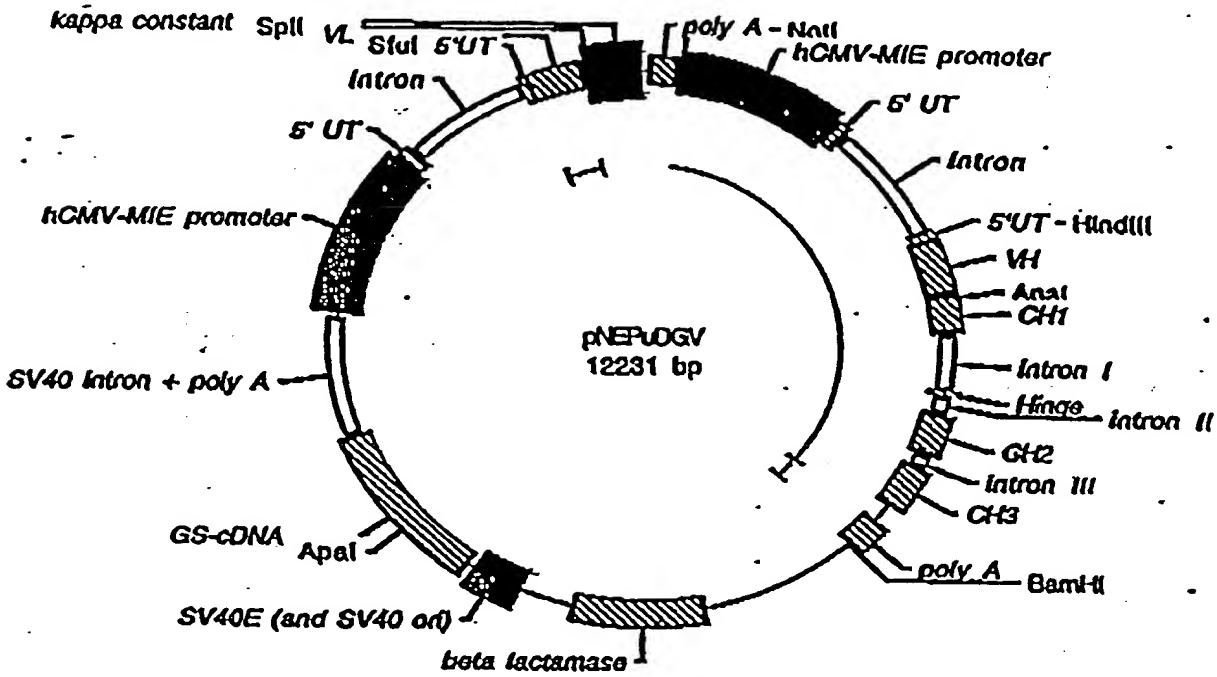

kappa constant
SpII
VL
StuI
5'UT
Intron
5' UT
hCMV-MIE promoter
SV40 intron + poly A
GS-cDNA
ApaI
SV40E (and SV40 ori)
beta lactamase
poly A - NotI
hCMV-MIE promoter
5' UT
Intron
5'UT - HindIII
VH
AnaI
CH1
Intron I
Hinge
Intron II
CH2
Intron III
CH3
poly A
BamHI
pNEPuDGV
12231 bp

FIG. 6C

```
ConVL1

EcoR1
GAA TTC
                                                        6

-19 (Leader)                                                    -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
      63

VL:


1                       10                      20
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
Thr
GAT ATC CAA ATG ACA CAA AGT CCT AGT AGT TTG AGT GCT AGT GTG GGA GAT CGG GTG
ACA    123

21                      30                      40
Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys
Pro
ATC ACA TGT CGG GCT AGT CAA AGT ATC AGT AAC TGT TTG GCT TGG TAT CAA CAA AAG
CCT    183

41                      50                      60
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro
Ser
GGA AAG GCT CCT AAG TTG TTG ATC TAT GCT GCT AGT AGT TTG GAG AGT GGA GTG CCT
AGT    243

61                      70                      80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Arg Phe Thr Leu Thr Ile Ser Ser Leu Gln
Pro
CGG TTC AGT GGA AGT GGA AGT GGA ACA CGG TTC ACC TTG ACC ATC AGT AGT TTG CAA
CCT    303

81                      90                      100
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Leu Pro Trp Thr Phe Gly
Gln
GAG GAT TTC GCT ACC TAT TAT TGT CAA CAA TAT AAC AGT TTG CCT TGG ACC TTC GGA
CAA    363

101
Gly Thr Lys Val Glu Ile Lys
GGA ACC AAG GTG GAG ATC AAG GAA TTC                              390
           Eco R1
```

FIG. 7A

```
ConVH1

EcoR1
GAA TTC                                                          6

-19 (Leader)                                                    -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala
Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC
CAA AGT GCC CAA GCA            63

VL:
1                    10                  20
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro
Gly Ala Ser Val Lys Val
CAG GTT CAG CTG GTG CAG TCT GGC GCT GAG GTG AAG AAG CCT
GGC GCT TCT GTG AAG GTG        123


21                   30                        35A 35B
40
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Ala Ile
Ser Trp Asn Trp Val Arg Gln Ala
TCT TGC AAG GCT TCT GGC TAC ACA TTC ACA TCT TAC GCT ATA
TCT TGG AAT TGG GTG AGG CAG GCT            189

41                   50                  60
Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Gly Asn
Gly Asp Thr Asn Tyr Ala
CCT GGC CAG GGC CTG GAG TGG ATG GGC TGG ATA AAT GGA AAT
GGA GAT ACA AAT TAC GCC        249

61                   70                  80
Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser
Thr Ser Thr Ala Tyr Met
CAG AAG TTC CAG GGA AGG GTG ACT ATA ACT GCT GAT ACT TCT
ACT TCT ACT GCT TAC ATG        309

81      82A 82B 82C                      90
       100
Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr
Cys Ala Arg Ala Pro Gly Tyr Gly Ser
GAG CTG TCT TCT CTG AGG TCT GAG GAT ACT GCT GTT TAC TAC
TGC GCT AGG GCT CCT GGC TAC GGC TCT        378

101                  110
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
GAT TAT TGG GGA CAG GGA ACA CTG GTT ACA GTT TCT TCT GAA TTC
      423
```

FIG. 7B

```
2CAVLCOL1
EcoR1                                                          6
GAA TTC                                                       -19  (Leader)
                                  -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA        63

1                                        10                                  20
Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu Val Ser Ala Gly Asp Arg Val Thr
AGT ATT GTG ATG ACC CAG ACT CCC AAA TTC CTG CTT GTA TCA GCA GGA GAC AGG GTT ACC    123

21                                       30                                  40
Ile Thr Ala Lys Ala Ser Gln Ser Val Ser Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro
ATA ACC GCT AAG GCC AGT CAG AGT GTG AGT AAT GAT GTA GCT TGG TAC CAA CAG AAA CCA    183

41                                       50                                  60
Gly Gln Ser Pro Lys Leu Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp
GGG CAG TCT CCT AAA CTG CTG ATA TAC TAT GCA TCC AAT CGC TAC ACT GGA GTC CCT GAT    243

61                                       70                                  80
Arg Phe thr Gly Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser thr Val Gln Ala
CGC TtC ACT GGC AGT GGA TAT GGG ACG GAT TTC ACT TTC ACC ATC AGC ACT GTG CAG GCT    303

81                                       90                                 100
Glu Asp Leu Ala Val Tyr Phe Ala Gln Gln Asp Tyr Ser Ser Pro Leu Thr Phe Gly Ala
GAA GAC CTG GCA GTT TAT TTC GCT CAG CAG GAT TAT AGC TCT CCG CTC ACG TTC GGT GCT    363

101
Gly Thr Lys Leu Glu Leu Lys
GGG ACC AAG CTG GAG CTG AAA GAA TTC                                            390
                        EcoR1
```

FIG. 8A

```
2CAVHCOL1
EcoR1
GAA TTC                                                                  6

-19 (Leader)                                                        -1
Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA      63

1                                        10                                 20
Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu Thr Val Lys Ile
CAG ATC CAG TTG GTG CAG TCT GGA CCT GAG CTG AAG AAG CCT GGA GAG ACA GTC AAG ATC   123

21                                       30                                 40
Ser Ala Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Lys Gln Ala
TCC GCT AAG GCT TCT GGG TAT ACC TTC ACA AAC TAT GGA ATG AAC TGG GTG AAG CAG GCT   183

41                                       50                                 60
Pro Gly Lys Gly Leu Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr
CCA GGA AAG GGT TTA AAG TGG ATG GGC TGG ATA AAC ACC TAC ACT GGA GAG CCA ACA TAT   243

61                                       70                                 80
Ala Asp Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
GCT GAT GAC TTC AAG GGA CGG TTT GCC TTC TCT TTG GAA ACC TCT GCC AGC ACT GCC TAT   303

81                                       90                                100
Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Ala Ala Arg Ala Tyr
TTG CAG ATC AAC AAC CTC AAA AAT GAG GAC ACG GCT ACA TAT TTC GCT GCA AGA GCC TAC   363

101
Tyr Gly Lys Tyr Phe Asp Tyr
TAT GGT AAA TAC TTT GAC TAC GAA TTC                                              390
```

FIG. 8B

2CAVHCOL1

VHC1 5'GAATTCATGGCTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGCCCAAAGTGCCC
AAGCACAGATCCAGTTGGTGCA 3'

VHC2 5'GTCTGGACCTGAGCTGAAGAAGCCTGGAGAGACAGTCAAGATCTCCGCTAAGGCTTC
TGGGTATACCTTCACAAACTAG 3'

VHC3 5'GAATGAACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGGGCTGGAT
AAACACCTACACTGGAGAGCCAACA 3'

VHC4 5'TATGCTGATGACTTCAAGGGACGGTTTGCCTTCTCTTTGGAAACCTCTGCCAGCACT
GCCTATTTGCAGATCAACACCT 3'

VHC5 5'CAAAAATGAGGACACGGCTACATATTTCGCTGCAAGAGCCTACTATGGTAAATAC
TTTGACTACGAATTC 3'

VHC6 5'GAATTCGTAGTCAAAGTATTTACCATAGTAGGCTCTTGCAGCAAATATG 3'

VHC7 5'TAGCCGTGTCCTCATTTTTGAGGTTGTTGATCTGCAAATAGGCAGTGCTGGCAGA
GGTTTCCAAAGAGAAGGCAAACCGT 3'

VHC8 5'CCCTTGAAGTCATCAGCATATGTTGGCTCTCCAGTGTAGGTGTTTATCCAGCCCAT
CCACTTTAAACCCTTTCCTGGAGC 3,

VHC9 5'CTGCTTCACCCAGTTCATTCCATAGTTTGTGAAGGTATACCCAGAAGCCTTAGCGG
AGATCTTGACTGTCTCTCCAGGCT 3'

VHC10 5'TCTTCAGCTCAGGTCCAGACTGCACCAACTGGATCTGTGCTTGGGCACTTTG GGC
AGCTGCCATCAGGAATAGCAAGGTCCACACCCAAGCCATGAATTC 3'

FIG. 9A

2CAVLCOL1

VLC1 5'AGTATTGTGATGACCCAGACTCCCAAATTCCTGCTTGTATCAGCAGGAGACAGGGTT
ACCATA 3'

VLC2 5'ACCTGCAAGGCCAGTCAGAGTGTGAGTAATGATGTAGCTTGGTACCAACAGAAAACC
AGGGCAG 3'

VLC3 5'TCTCCTAAACTGCTGATATACTATGCATCCAATCGCTACACTGGAGTCCCTGATCGCT
TCACTGGCAGT 3'

VLC4 5'GGATATGGGACGGATTTCACTTTCACCATCAGCACTGTGCAGGCTGAAGACCTGGCA
GTTTAT 3'

VLC5 5'TTCTGYCAGCAGGATTATAGCTCTCCGCTCACGTTCGGTGCTGGGACCAAGCTGGAG
CTGAAAGAATTC 3'

VLC6 5'GAATTCTTTCAGCTCCAGCTTGGTCCCAGCACCGAACGTGAGCGGAGAGCTATAATC
CTGCTGACAGAAATAAACTGC 3'

VLC7 5'CAGGTCTTCAGCCTGCACAGTGCTGATGGTGAAAGTGAAATCCGTCCCATATCCA
CTGCCAGT 3'

VLC8 5'GAAGCGATCAGGGACTCCAGTGTAGCGATTGGATGCATAGTATATCAGCAGTTTAG
GAGACTGCCCTGG 3'

VLC9 5'TTTCTGTTGGTACCAAGCTACATCATTACTCACACTCTGACTGGCCTTGCAGGTTA
TGGTAAC 3'

VLC10 5'CCTGTCTCCTGCTGATACAAGCAGGAATTTGGGAGTCTGGGTCATCACAATACTT
GCTTGGGC 3'

VLC11 5'TTCGCTCAGCAGGATTATAGCTCTCCGCTCACGTTCGGTGCTGGGACCAAGCTGG
AGCTGAAAGAATC 3'

VLC12 5'GAATTCTTTCAGCTCCAGCTTGGTCCCAGCACCGAACGTGAGCGGAGAGCTATAA
TCCTGCTGAGCGAAATAAACTGC 3'

FIG. 9B

ConVL1

Leader Sequence

L1 5'GAATTCATGGCTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGCCCAAAGTGCCC
AAGCA 3'

L2 5'ACTTTGGGCAGCTGCCATCAGGAATAGCAAGGTCCACACCCAAGCCATGAATTC 3'

BKLC1 5'GATATCCAAATGACACAAAGTCCTAGTAGTTTGAGTGCTAGTGTGGGAGATCG
GGTGATCACA 3'

BKLC2 5' TGTCGGGCTAGTCAAAGTATCAGTAACTATTTGGCTTGGTATCAACAAAAGCCT
GGAAAGGCTCCTAAGTTGTTGATC 3'

BKLC3 5' TATGCTGCTAGTAGTTTGGAGAGTGGAGTGCCTAGTCGGTTCAGTGGA 3'

BKLC4 5' AGTGGAAGTGGAACACGGTTCACCTTGACCATCAGTAGTTTGCAACCTGAGGA
TTCGCTACCTATTAT 3'

BKLC5 5' TGTCAACAATATAACAGTTTGCCTTGGACCTTCGGACAAGGAACCAAGGTGGA
GATCAAGGAATTC3'

BKLC6 5' GAATTCCTTGATCTCCACCTTGGTTCCTTGTCCGAAGGTCCAAGGCAAACTGTTA
TATTGTTGACAATAATAGGT3'

BKLC7 5'AGCGAAATCCTCAGGTTGCAAACTACTGATGGTCAAGGTGAACCGTGTTCCACTT
CCACTTCCACTGAA3'

BKLC8 5'CCGACTAGGCACTCCACTCTCCAAACTACTAGCAGCATAGATCAACAA 3'

BKLC9 5' CTTAGGAGCCTTTCCAGGCTTTTGTTGATACCAAGCCAAATAGTTACTGATACT
TTGACTAGCCCGACATGTGATTGT 3'

BKLC10 5'CACCCGATCTCCCACACTAGCACTCAAACTACTAGGACTTTGTGTCATTTGGA
TATCTTGCTTGGGC3'

BKLCDR12 5'TGTCGGCCTCCTGGCTTCTCTCCTTTCAGGTTGGCTTGGTATCAACAAAAGC
CTGGAAAGGCTCCTAAGTTGTTGATC 3'

BKLCDR19 5'CTTAGGAGCCTTTCCAGGCTTTTGTTGATACCAAGCCAACCTGAAAGGAGA
GAAGCCAGGAGGCCGACATGTGATTGT3'

BKLCDR23 5'TATCCTGGCTTCTCTCCTTTCAGGGGAGTGCCTAGTCGGTCAGTGGA 3'

BKLCDR28 5'CCGACTAGGCACTCCCCTGAAAGGAGAGAAGCCAGGATAGATCAACAA 3'

BKLCDR35 5'TGTAGGCCTCCTGGCTTCTCTCCTTTCAGGTTCGGACAAGGAACCAAGGTGG
AGATCAAG 3'

BKLCDR36 5' GAATTCCTTGATCTCCACCTTGGTTCCTTGTCCGAACCTGAAAGGAGAGAA
GCCAGGAGGCCTACAATAATAGGT 3'

FIG. 9C

ConVH1

BKHC1 5'GAATTCATGGCTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGCCCAAAGTG
CCCAAGCACAGATCCAGTTGGTGCAGTCTG 3'

BKHC2 5'GCGCTGAGGTGAAGAAGCCTGGCGCTTCTGTGAAGGTGTCTTGCAAGGCTTCT
GGCTACATTCACATCTTACGCTATATCTTG 3'

BKHC3 5'GAATTGGGTGAGGCAGGCTCCTGGCCAGGGCCTGGAGTGGATGGGCTGGATAAAT
GGAAATGGAGATACAATTACGCCCAGAAG 3'

BKHC4 5'TTCCAGGGAAGGGTTACTATAACTGCTGATACTTCTACTTCTACTGCTTACATGG
AGCTGTCTTCTCTGAGGTCTGAGGATACT 3'

BKHC5 5'GCTGTTTACTACTGCGCTAGGGCTCCTGGCTACGGCTCTGATTATTGGGGACA
GGGAACACTGGTTACAGTTTCTTTCTGAATTC 3'

BKHC6 5'GAATTCAGAAGAAACTGTAACCAGTGTTCCCTGTCCCCAATAATCAGAGCCGTA
GCCAGGAGCC 3'

BKHC7 5' CTAGCGCAGTAGTAAACAGCAGTATCCTCAGACCTCAGAGAAGACAGCTCCAT
GTAAGCAGTAGAAGTAGAAGTATCAGCAGTT 3'

BKHC8 5'ATAGTAACCCTTCCCTGGAACTTCTGGGCGTAATTTGTATCTCCATTTCCATTT
ATCCAGCCCATCCACTCCAGGCCCTGGCCAG 3'

BKHC9 5'GAGCCTGCCTCACCCAATTCCAAGATATAGCGTAAGATGTGAATGTGTAGCCA
GAAGCCTTGCAAGACACCTTCACAGAAGCGCC 3'

BKHC10 5'AGGCTTCTTCACCTCAGCGCCAGACTGCACCAGCTGAACCTGTGCTTGGGCACT
TTGGGCAGCTGCCATCAGGAATAGCAAGGTCCACACCCAAGCCATGAATTC 3'

BKHCDR42 5'GCGCTGAGGTGAAGAAGCCTGGCGCTTCTGTGAAGGTGTCTTGCAAGGC
TTCTGGCTACACATTCACA 3'

BKHDR43 5'CAGGTGGGTGAGGCAGGCTCCTGGCCAGGGCCTGGAGTGGATGGGCTGGAT
AAATGGAGATACAAATTACGCCCAGAAG 3'

BKHDR49 5'GAGCCTGCCTCACCCACCTGAAAGGAGAGAAGCCAGGTGTGAATGTGTA
GCCAGAAGCCTTGCAAGACACCTTCACAGAAGCGCC 3'

BKHDR53 5'GAATTGGGTGAGGCAGGCTCCTGGCCAGGGCCTGGAGTGGATGGGCTGGATA
AATGGAAGGCCTCCTGGCTTCTCTCCTTTCAGG 3'

BKHDR58 5'ATAGTAACCCTTCCCTGGAACCTGAAAGGAGAGAAGCCAGGAGGCCTTC
CATTTATCCAGCCCATCCACTCCAGGCCCTGGCCAG 3'

FIG. 9D

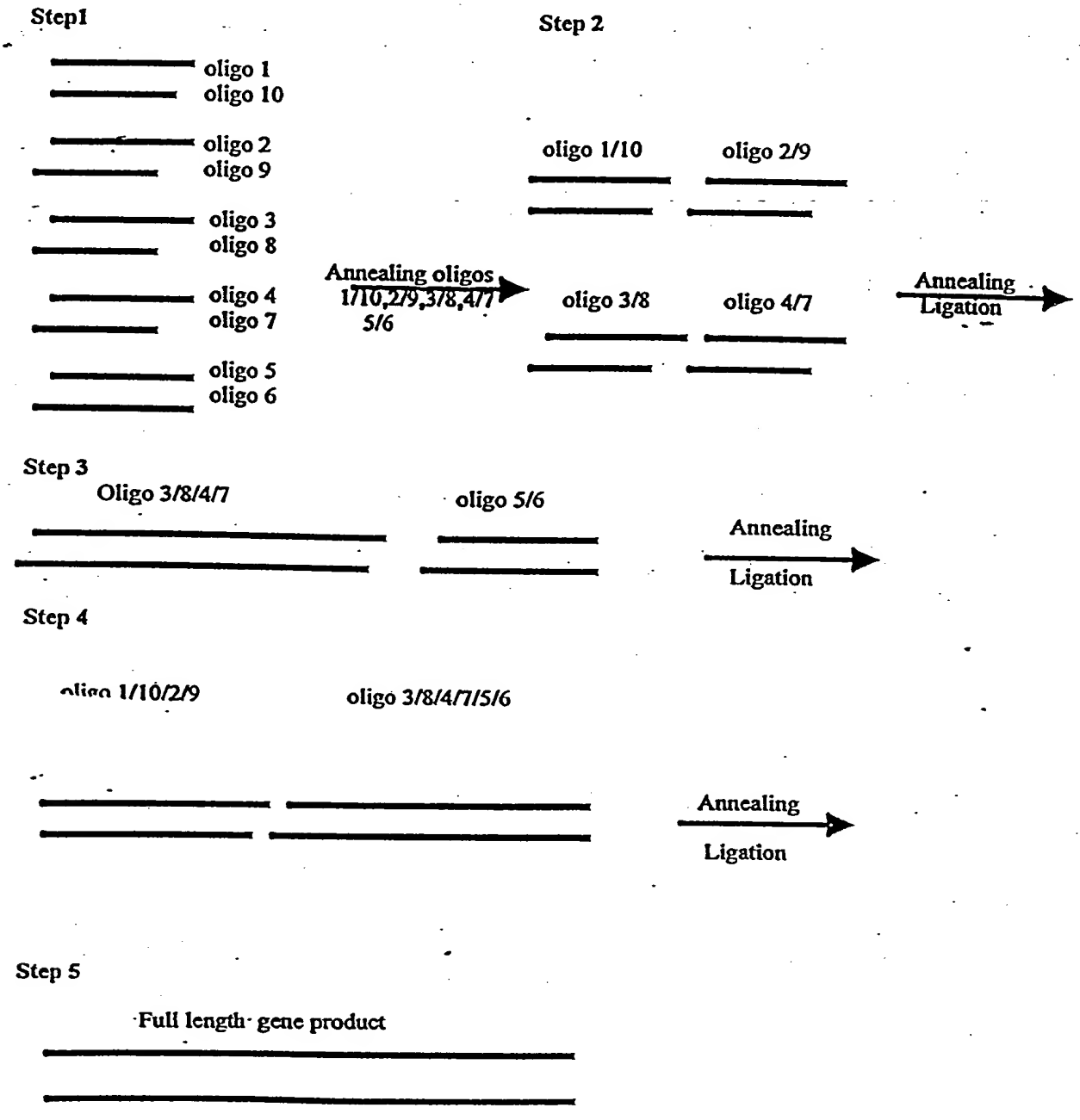

Step1
oligo 1
oligo 10
oligo 2
oligo 9
oligo 3
oligo 8
oligo 4
oligo 7
oligo 5
oligo 6
Annealing oligos 1/10,2/9,3/8,4/7 5/6
Step 2
oligo 1/10
oligo 2/9
oligo 3/8
oligo 4/7
Annealing
Ligation
Step 3
Oligo 3/8/4/7
oligo 5/6
Annealing
Ligation
Step 4
oligo 1/10/2/9
oligo 3/8/4/7/5/6
Annealing
Ligation
Step 5
Full length gene product

FIG. 10

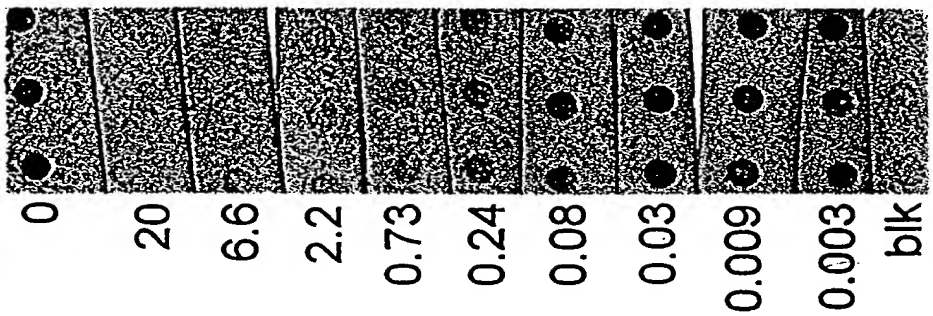
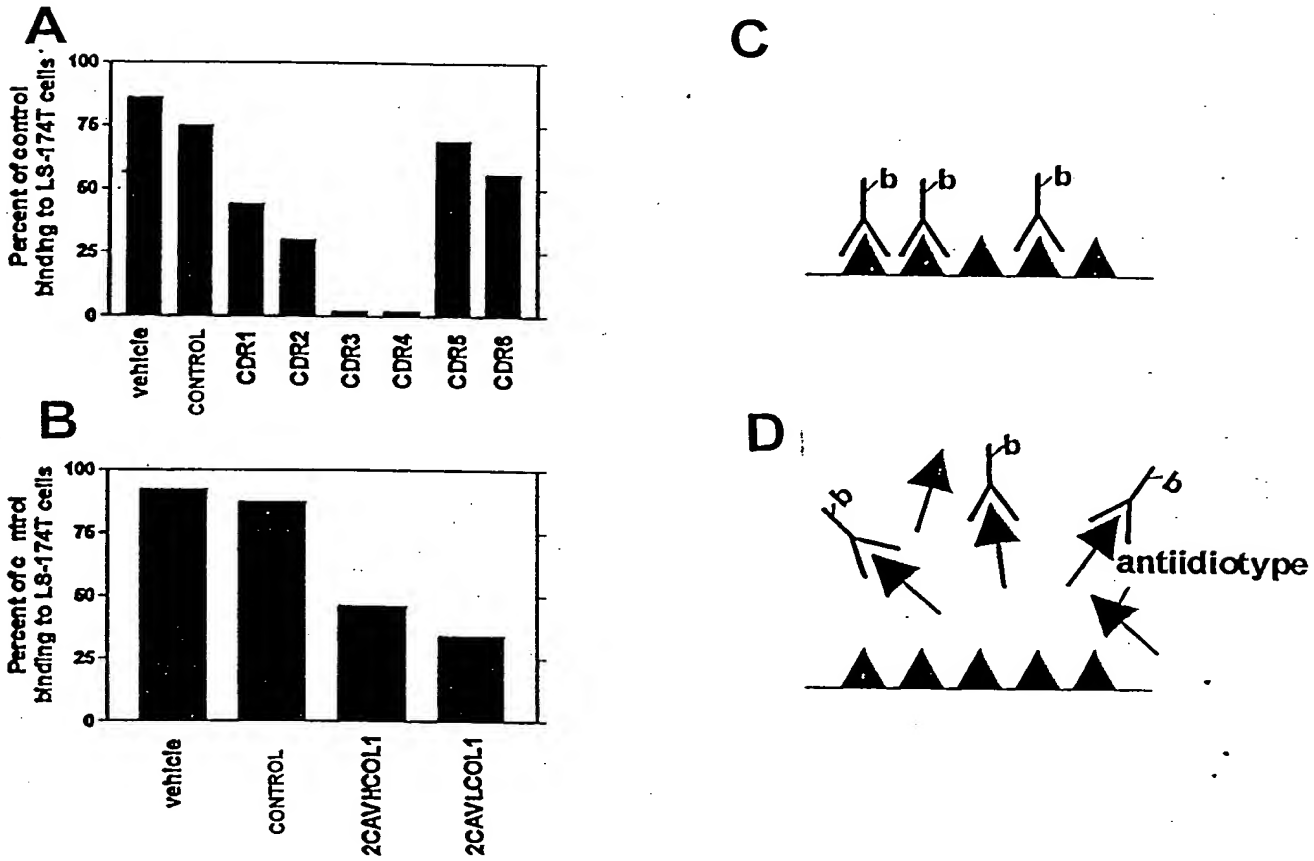


FIG. 11


A
Percent of control binding to LS-174T cells
100
75
50
25
0
vehicle
CONTROL
CDR1
CDR2
CDR3
CDR4
CDR5
CDR6
B
Percent of control binding to LS-174T cells
100
75
50
25
0
vehicle
CONTROL
2CAVHCOL1
2CAVLCOL1
C
b
b
b
D
b
b
b
antiidiotype

FIGS. 12A-D


oligo 1
1 GACATTGTGA TGTCACAGTC TCCATCCTCC CTAGCTGTGT CAGTTGGAGA
oligo 2
51 GAAGGTTACT ATGAGCTGCA AGTCCAGTCA GAGCCTTTTA TATAGTAGCA
oligo 8
101 ATCAAAAGAT CTACTTGGCC TGGTACCAGC AGAAACCAGG GCAGTCTCCT
oligo 3
151 AAACTGCTGA TTTACTGGGC ATCCACTAGG GAATCTGGGG TCCCTGATCG
oligo 7
oligo 4
201 CTTCACAGGC GGTGGATCTG GGACAGATTT CACTCTCACC ATCAGCAGTG
251 TGAAGGCTGA AGACCTGGCA GTTTATTACT GTCAGCAATA TTATAGATAT
oligo 6
301 CCTCGGACGT TCGGTGGAGG CACCAAGCTG GAAATCAAAC GG
oligo 5

FIG. 13

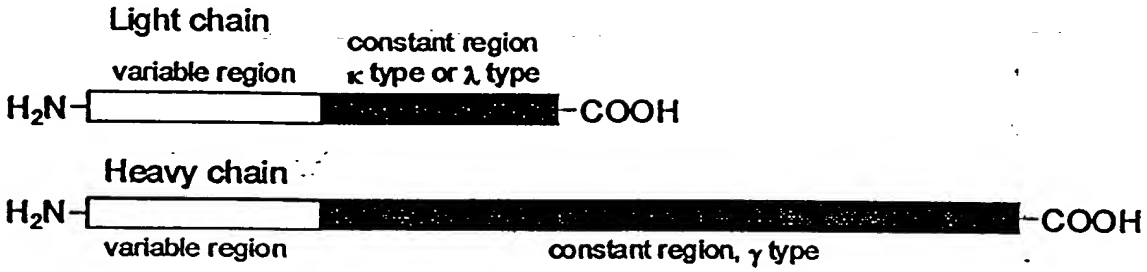

Light chain
variable region
constant region
κ type or λ type
H2N
COOH
Heavy chain
H2N
COOH
variable region
constant region, γ type

FIG. 1

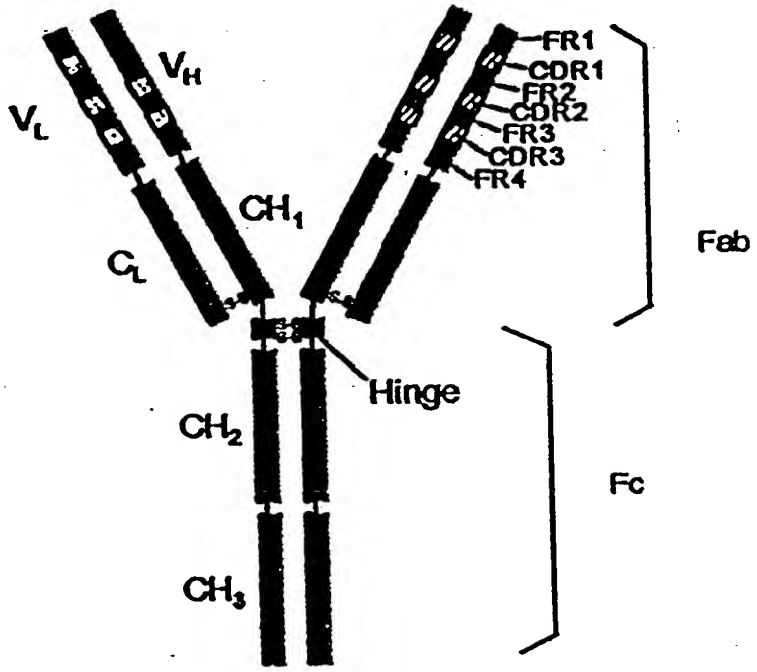


FIG. 2

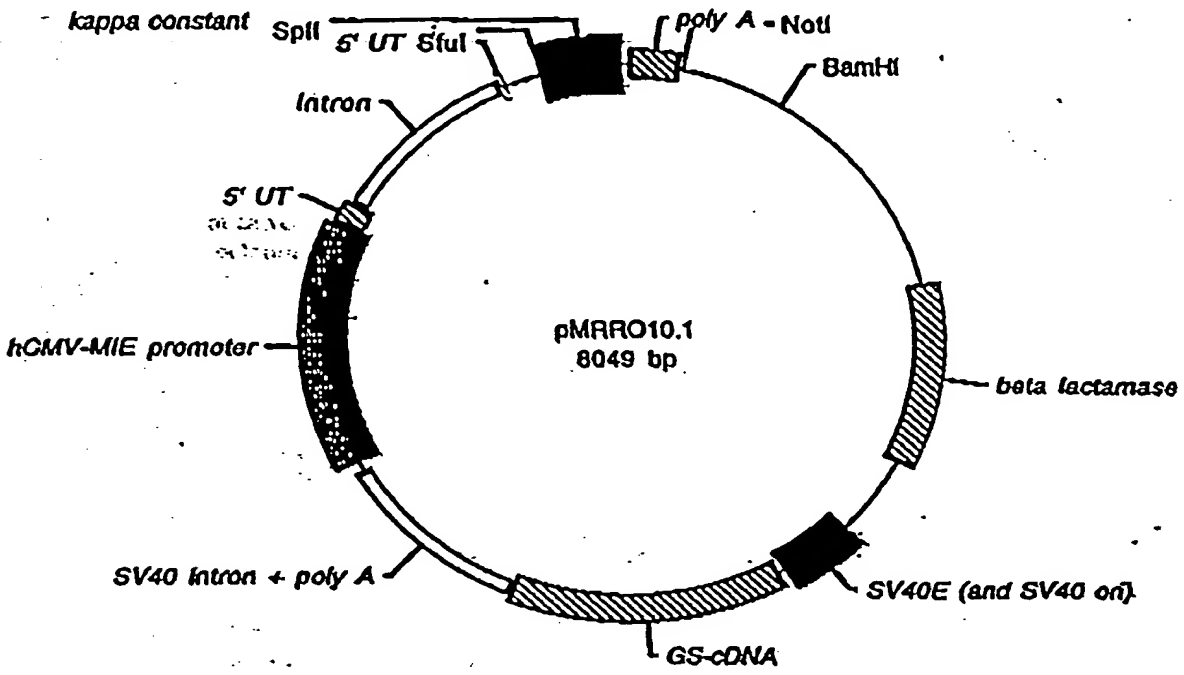

kappa constant
SpII
5' UT
SfuI
poly A - NotI
BamHI
Intron
5' UT
hCMV-MIE promoter
pMRRO10.1
8049 bp
beta lactamase
SV40 intron + poly A
SV40E (and SV40 ori)
GS-cDNA

FIG. 3A

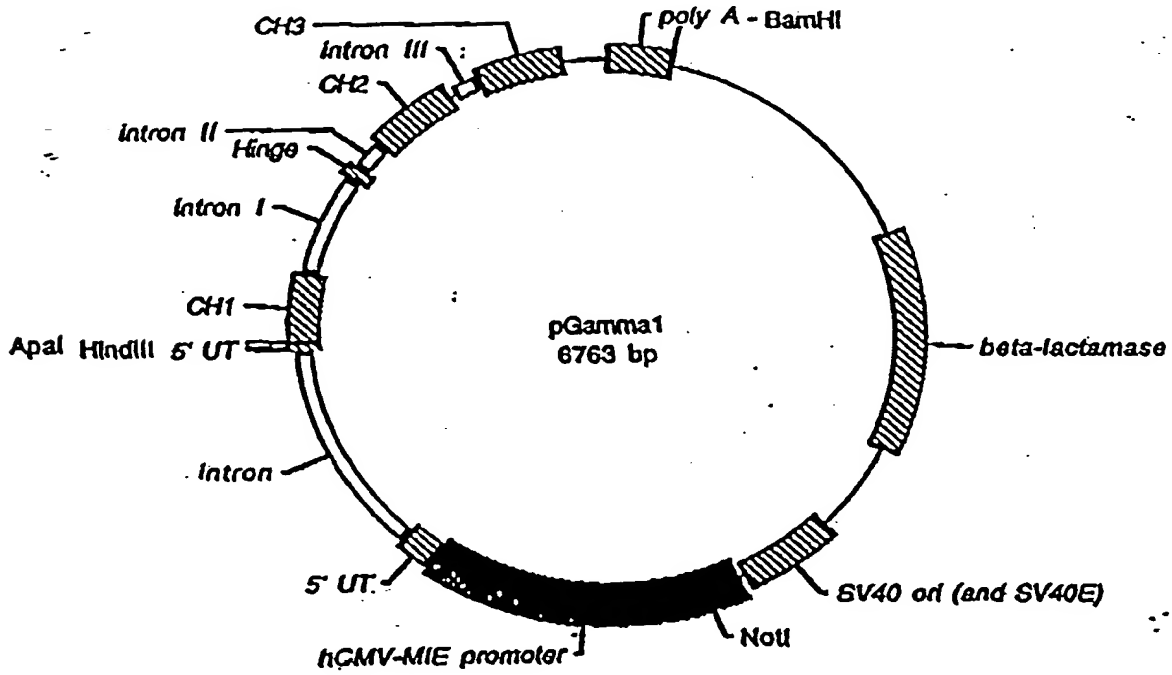

CH3
Intron III
CH2
Intron II
Hinge
Intron I
CH1
ApaI HindIII 5' UT
Intron
5' UT
hCMV-MIE promoter
NotI
SV40 ori (and SV40E)
beta-lactamase
poly A - BamHI
pGamma1
6763 bp

FIG. 3B

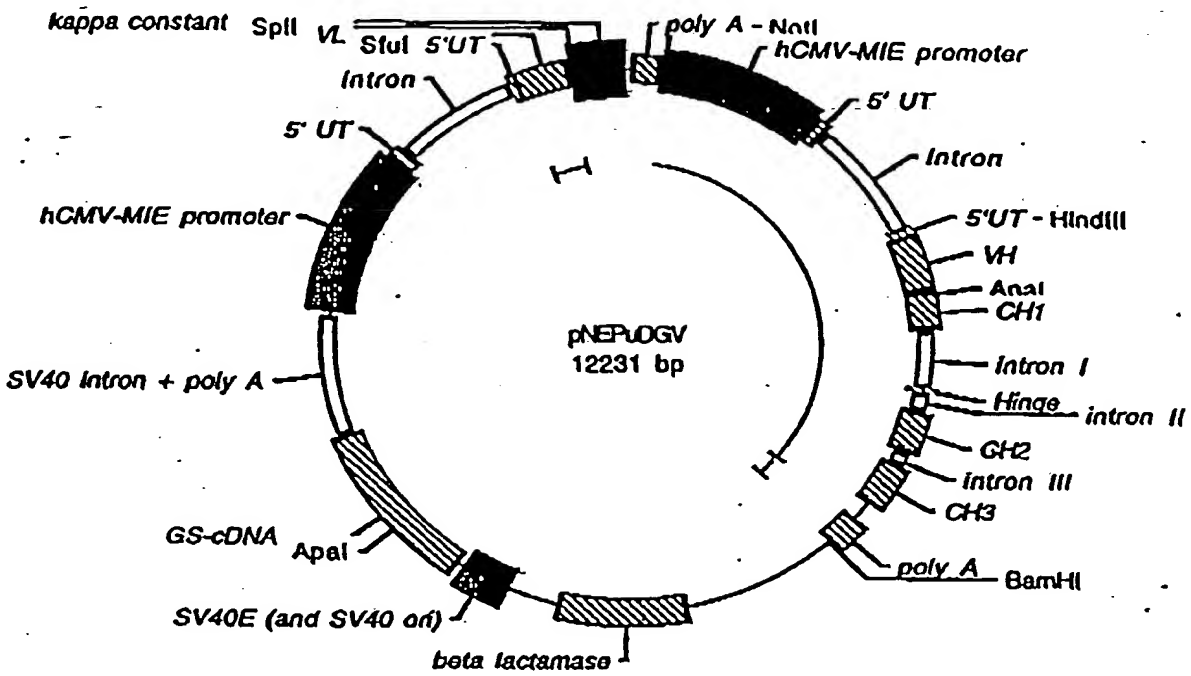

kappa constant
SpII
VL
StuI
5'UT
poly A - NotI
hCMV-MIE promoter
Intron
5' UT
5' UT
Intron
hCMV-MIE promoter
5'UT - HindIII
VH
ApaI
CH1
pNEPuOGV
12231 bp
Intron I
Hinge
intron II
CH2
Intron III
CH3
SV40 Intron + poly A
poly A
BamHI
GS-cDNA
ApaI
SV40E (and SV40 ori)
beta lactamase

FIG. 3C

ConVL1

EcoR1
GAA TTC

6

-19 (Leader) -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
63

V_L:

1 10 20
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
Thr
GAT ATC CAA ATG ACA CAA AGT CCT AGT AGT TTG AGT GCT AGT GTG GGA GAT CGG GTG
ACA 123

21 30 40
Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys
Pro
ATC ACA TGT CGG GCT AGT CAA AGT ATC AGT AAC TGT TTG GCT TGG TAT CAA CAA AAG
CCT 183

41 50 60
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro
Ser
GGA AAG GCT CCT AAG TTG TTG ATC TAT GCT GCT AGT AGT TTG GAG AGT GGA GTG CCT
AGT 243

61 70 80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Arg Phe Thr Leu Thr Ile Ser Ser Leu Gln
Pro
CGG TTC AGT GGA AGT GGA AGT GGA ACA CGG TTC ACC TTG ACC ATC AGT AGT TTG CAA
CCT 303

81 90 100
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Leu Pro Trp Thr Phe Gly
Gln
GAG GAT TTC GCT ACC TAT TAT TGT CAA CAA TAT AAC AGT TTG CCT TGG ACC TTC GGA
CAA 363

101
Gly Thr Lys Val Glu Ile Lys
GGA ACC AAG GTG GAG ATC AAG GAA TTC 390
Eco R1

FIG. 4A

BKCDR1

EcoR1
GAA TTC 6

-19 (Leader) -1
Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
63

V_L:

1 10 20
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
Thr
GAT ATC CAA ATG ACA CAA AGT CCT AGT AGT TTG AGT GCT AGT GTG GGA GAT CAA GTG
ACA 123

21 30 40
Ile Thr Cys Arg Pro Pro Gly Phe Ser Pro Phe Arg Leu Ala Trp Tyr Gln Gln Lys
Pro
ATC ACA TGT CAA CCT CCT GGC TTC TCT CCT TTC AGG TTG GCT TGG TAT CCA CAA AAG
CCT 183

41 50 60
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro
Ser
GGa AGG GCT CCT AAG TTG TTG ATC TAT GCT GCT AGT AGT TTG GAG AGT GGA GTG CCT
AGT 243

61 70 80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Arg Phe Thr Leu Thr Ile Ser Ser Leu Gln
Pro
CGG TTC AGT GGA AGT GGA AGT GGA ACA CGG TTC ACC TTG ACC ATC AGT AGT TTG CAA
CCT 303

81 90 100
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Leu Pro Trp Thr Phe Gly
Gln
GAG GAT TTC GCT ACC TAT TAT TGT CAA CAA TAT AAC AGT TTG CCT TGG ACC TTC GGA
CAA 363

101
Gly Thr Lys Val Glu Ile Lys
GGA ACC AAG GTG GAG ATC AAG GAA TTC 390
EcoR1

FIG. 4B

BKCDR2

EcoR1
GAA TTC 6

-19 (Leader) -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
63

V_L:

1 10 20
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
Thr
GAT ATC CAA ATG ACA CAA AGT CCT AGT AGT TTG AGT GCT AGT GTG GGA GAT CAA GTG
ACA 123

21 30 40
Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys
Pro
ATC ACA TGT CGG GCT AGT CAA AGT ATC AGT AAC TAT TTG GCT TGG TAT CAA CAA AAG
CCT 183

41 50 60
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Pro Gly Phe Ser Pro Phe Arg Gly Val Pro
Ser
GGA AAG GCT CCT AAG TTG TTG ATC TAT CCT GGC TTC TCT CCT TTC CGG GGA GTG CCT
AGT 243

61 70 80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Arg Phe Thr Leu Thr Ile Ser Ser Leu Gln
Pro
CGG TTC AGT GGA AGT GGA AGT GGA ACA CGG TTC ACC TTG ACC ATC AGT AGT TTG CAA
CCT 303

81 90 100
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Leu Pro Trp Thr Phe Gly
Gln
GAG GAT TTC GCT ACC TAT TAT TGT CAA CAA TAT AAC AGT TTG CCT TGG ACC TTC GGA
CAA 363

101
Gly Thr Lys Val Glu Ile Lys
GGA ACC AAG GTG GAG ATC AAG GAA TTC 390
EcoR1

FIG. 4C

```
BKCDR3

EcoR1
GAA TTC                                                    6

-19 (Leader)                                                -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
63

VL:
1                         10                        20
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
Thr
GAT ATC CAA ATG ACA CAA AGT CCT AGT AGT TTG AGT GCT AGT GTG GGA GAT CAA GTG
ACA   123

21                        30                        40
Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys
Pro
ATC ACA TGT CGG GCT AGT CAA AGT ATC AGT AAC TAT TTG GCT TGG TAT CAA CAA AAG
CCT   183

41                        50                        60
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro
Ser
GGA AAG GCT CCT AAG TTG TTG ATC TAT GCT GCT AGT AGT TTG GAG AGT GGA GTG CCT
AGT   243

61                        70                        80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Arg Phe Thr Leu Thr Ile Ser Ser Leu Gln
Pro
CGG TTC AGT GGA AGT GGA AGT GGA ACA CGG TTC ACC TTG ACC ATC AGT AGT TTG CAA
CCT   303

81                        90                        100
Glu Asp Phe Ala Thr Tyr Tyr Cys Arg Pro Pro Gly Phe Ser Pro Phe Arg Phe Gly
Gln
GAG GAT TTC GCT ACC TAT TAT TGT AGG CCT CCT GGC TTC TCT CCT TTC AGG TTC GGA
CAA   363

101
Gly Thr Lys Val Glu Ile Lys
GGA ACC AAG GTG GAG ATC AAG GAA TTC                                  390
            EcoR1
```

FIG. 4D

```
ConVH1

EcoR1
GAA TTC                                                          6

-19 (Leader)                                                    -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala
Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC
CAA AGT GCC CAA GCA            63

VL:
1                   10                  20
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro
Gly Ala Ser Val Lys Val
CAG GTT CAG CTG GTG CAG TCT GGC GCT GAG GTG AAG AAG CCT
GGC GCT TCT GTG AAG GTG        123


21                  30                      35A 35B
40
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Ala Ile
Ser Trp Asn Trp Val Arg Gln Ala
TCT TGC AAG GCT TCT GGC TAC ACA TTC ACA TCT TAC GCT ATA
TCT TGG AAT TGG GTG AGG CAG GCT            189

41                  50                  60
Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Gly Asn
Gly Asp Thr Asn Tyr Ala
CCT GGC CAG GGC CTG GAG TGG ATG GGC TGG ATA AAT GGA AAT
GGA GAT ACA AAT TAC GCC        249

61                  70                  80
Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser
Thr Ser Thr Ala Tyr Met
CAG AAG TTC CAG GGA AGG GTG ACT ATA ACT GCT GAT ACT TCT
ACT TCT ACT GCT TAC ATG        309

81      82A 82B 82C                 90
       100
Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr
Cys Ala Arg Ala Pro Gly Tyr Gly Ser
GAG CTG TCT TCT CTG AGG TCT GAG GAT ACT GCT GTT TAC TAC
TGC GCT AGG GCT CCT GGC TAC GGC TCT        378

101                 110
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
GAT TAT TGG GGA CAG GGA ACA CTG GTT ACA GTT TCT TCT GAA TTC
      423
```

FIG. 4E

```
BKCDR4

EcoR1
GAA TTC                                                          6

-19 (Leader)                                                    -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
      6 3

VL:

1                          10                          20
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys
Val
CAG GTT CAG CTG GTG CAG TCT GGC GCT GAG GTG AAG AAG CCT GGC GCT TCT GTG AAG
GTG          123

21                         30                          35A 35B                 40
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Pro Gly Phe Ser Pro Phe Arg Trp Val
Arg Gln Ala
TCT TGC AAG GCT TCT GGC TAC ACA TTC ACA CCT GGC TTC TCT CCT TTC AGG TGG GTG
AGG CAG GCT 189

41                         50                          60
Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Gly Asn Gly Asp Thr Asn Tyr
Ala
CCT GGC CAG GGC CTG GAG TGG ATG GGC TGG ATA AAT GGA AAT GGA GAT ACA AAT TAC
GCC          249


61                         70                          80
Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
Met
CAG AAG TTC CAG GGA AGG GTG ACT ATA ACT GCT GAT ACT TCT ACT TCT ACT GCT TAC
ATG          309

81       82A 82B 82C                                90                      100
Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ala Pro
Gly Tyr Gly Ser
GAG CTG TCT TCT CTG AGG TCT GAG GAT ACT GCT GTT TAC TAC TGC GCT AGG GCT CCT
GGC TAC GGC TCT    378

101                        110
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
GAT TAT TGG GGA CAG GGA ACA CTG GTT ACA GTT TCT TCT GAA TTC
       423
```

FIG. 4F

BKCDR5

EcoR1
GAA TTC 6

-19 (Leader) -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
63

V_L:

1 10 20
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys
Val
CAG GTT CAG CTG GTG CAG TCT GGC GCT GAG GTG AAG AAG CCT GGC GCT TCT GTG AAG
GTG 123

21 30 35A 35B 40
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Ala Ile Ser Trp Asn Trp Val
Arg Gln Ala
TCT TGC AAG GCT TCT GGC TAC ACA TTC ACA TCT TAC GCT ATA TCT TGG AAT TGG GTG
AGG CAG GCT 189

41 50 60
Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Gly Arg Pro Pro Gly Phe Ser
Pro
CCT GGC CAG GGC CTG GAG TGG ATG GGC TGG ATA AAT GGA AGG CCT CCT GGC TTC TCT
CCT 249

61 70 80
Phe Arg Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
Met
TTC AAG TTC CAG GGA AGG GTG ACT ATA ACT GCT GAT ACT TCT ACT TCT ACT GCT TAC
ATG 309

81 82A 82B 82C 90 100
Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ala Pro
Gly Tyr Gly Ser
GAG CTG TCT TCT CTG AGG TCT GAG GAT ACT GCT GTT TAC TAC TGC GCT AGG GCT CCT
GGC TAC GGC TCT 378

101 110
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
GAT TAT TGG GGA CAG GGA ACA CTG GTT ACA GTT TCT TCT GAA TTC
423

FIG. 4G

BKCDR6

EcoR1
GAA TTC 6

-19 (Leader) -1
Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
63

V_L:

1 10 20
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys
Val
CAG GTT CAG CTG GTG CAG TCT GGC GCT GAG GTG AAG AAG CCT GGC GCT TCT GTG AAG
GTG 123

21 30 35A 35B 40
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Ala Ile Ser Trp Asn Trp Val
Arg Gln Ala
TCT TGC AAG GCT TCT GGC TAC ACA TTC ACA TCT TAC GCT ATA TCT TGG AAT TGG GTG
AGG CAG GCT 189

41 50 60
Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Gly Asn Gly Asp Thr Asn Tyr
Ala
CCT GGC CAG GGC CTG GAG TGG ATG GGC TGG ATA AAT GGA AAT GGA GAT ACA AAT TAC
GCC 249

61 70 80
Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
Met
CAG AAG TTC CAG GGA AGG GTG ACT ATA ACT GCT GAT ACT TCT ACT TCT ACT GCT TAC
ATG 309

81 82A 82B 82C 90 100
Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Pro Pro
Gly Phe Ser Pro
GAG CTG TCT TCT CTG AGG TCT GAG GAT ACT GCT GTT TAC TAC TGC GCT AGG CTT CCT
GGC TTC TCT CCT 378

101 110
Phe Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
TTC AGG TGG GGA CAG GGA ACA CTG GTT ACA GTT TCT TCT GAA TTC
423

FIG. 4H

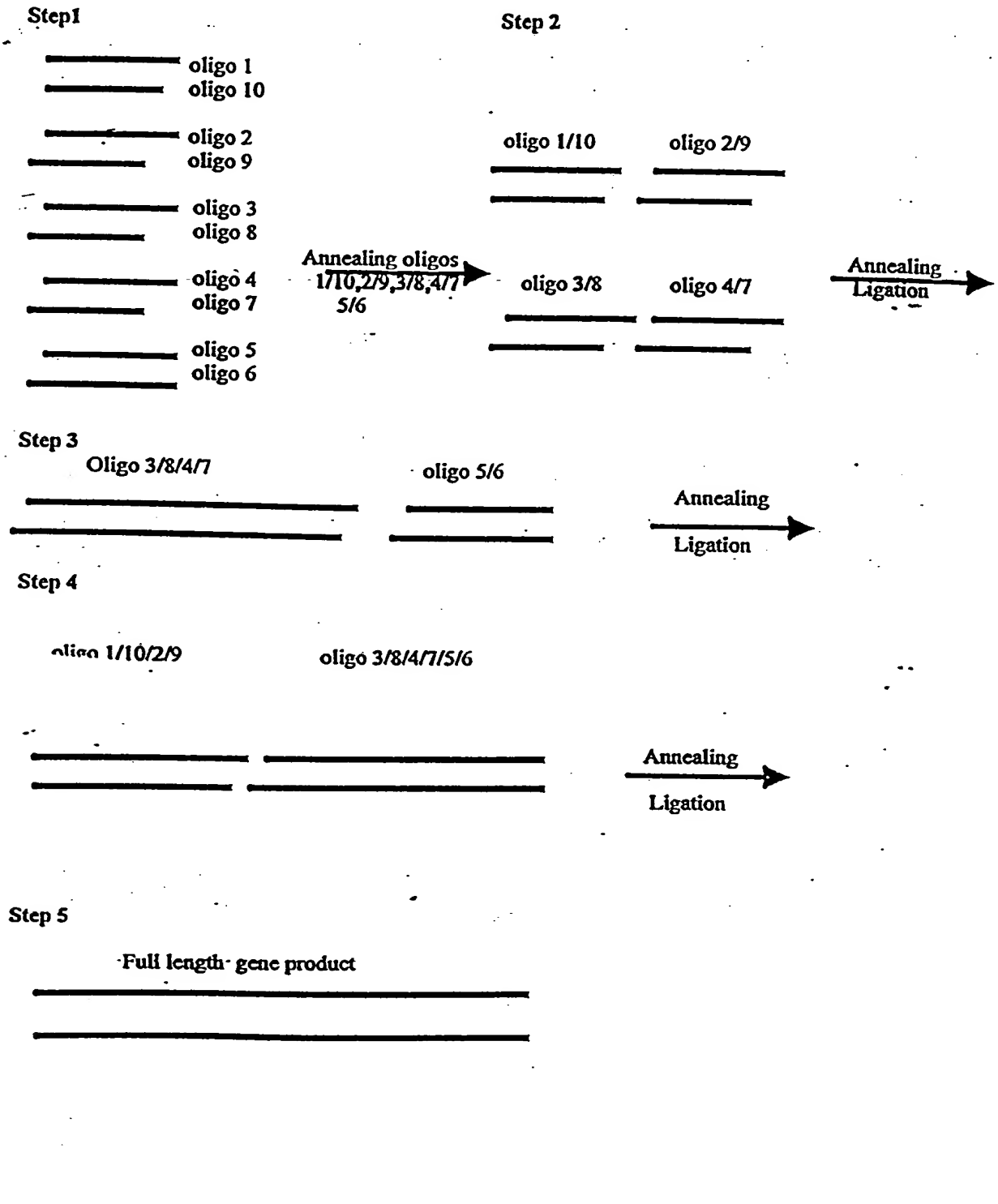

Step1
oligo 1
oligo 10
oligo 2
oligo 9
oligo 3
oligo 8
oligo 4
oligo 7
oligo 5
oligo 6
Annealing oligos
1/10,2/9,3/8,4/7
5/6
Step 2
oligo 1/10
oligo 2/9
oligo 3/8
oligo 4/7
Annealing
Ligation
Step 3
Oligo 3/8/4/7
oligo 5/6
Annealing
Ligation
Step 4
oligo 1/10/2/9
oligo 3/8/4/7/5/6
Annealing
Ligation
Step 5
Full length gene product

FIG. 5

ConVL1

Leader Sequence

L1 5'GAATTCATGGCTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGCCCAAAGTGCCC
AAGCA 3'

L2 5'ACTTTGGGCAGCTGCCATCAGGAATAGCAAGGTCCACACCCAAGCCATGAATTC 3'

BKLC1 5'GATATCCAAATGACACAAAGTCCTAGTAGTTTGAGTGCTAGTGTGGGAGATCG
GGTGATCACA 3'

BKLC2 5' TGTCGGGCTAGTCAAAGTATCAGTAACTATTTGGCTTGGTATCAACAAAAGCCT
GGAAAGGCTCCTAAGTTGTTGATC 3'

BKLC3 5' TATGCTGCTAGTAGTTTGGAGAGTGGAGTGCCTAGTCGGTTCAGTGGA 3'

BKLC4 5' AGTGGAAGTGGAACACGGTTCACCTTGACCATCAGTAGTTTGCAACCTGAGGA
TTCGCTACCTATTAT 3'

BKLC5 5' TGTCAACAATATAACAGTTTGCCTTGGACCTTCGGACAAGGAACCAAGGTGGA
GATCAAGGAATTC3'

BKLC6 5' GAATTCCTTGATCTCCACCTTGGTTCCTTGTCCGAAGGTCCAAGGCAAACTGTTA
TATTGTTGACAATAATAGGT3'

BKLC7 5'AGCGAAATCCTCAGGTTGCAAACTACTGATGGTCAAGGTGAACCGTGTTCCACTT
CCACTTCCACTGAA3'

BKLC8 5'CCGACTAGGCACTCCACTCTCCAAACTACTAGCAGCATAGATCAACAA 3'

BKLC9 5' CTTAGGAGCCTTTCCAGGCTTTTGTTGATACCAAGCCAAATAGTTACTGATACT
TTGACTAGCCCGACATGTGATTGT 3'

BKLC10 5'CACCCGATCTCCCACACTAGCACTCAAACTACTAGGACTTTGTGTCATTTGGA
TATCTTGCTTGGGC3'

BKLCDR12 5'TGTCGGCCTCCTGGCTTCTCTCCTTTCAGGTTGGCTTGGTATCAACAAAAGC
CTGGAAAGGCTCCTAAGTTGTTGATC 3'

BKLCDR19 5'CTTAGGAGCCTTTCCAGGCTTTTGTTGATACCAAGCCAACCTGAAAGGAGA
GAAGCCAGGAGGCCGACATGTGATTGT3'

BKLCDR23 5'TATCCTGGCTTCTCTCCTTTCAGGGGAGTGCCTAGTCGGTCAGTGGA 3'

BKLCDR28 5'CCGACTAGGCACTCCCCTGAAAGGAGAGAAGCCAGGATAGATCAACAA 3'

BKLCDR35 5'TGTAGGCCTCCTGGCTTCTCTCCTTTCAGGTTCGGACAAGGAACCAAGGTGG
AGATCAAG 3'

BKLCDR36 5' GAATTCCTTGATCTCCACCTTGGTTCCTTGTCCGAACCTGAAAGGAGAGAA
GCCAGGAGGCCTACAATAATAGGT 3'

FIG. 6A

ConVH1

BKHC1 5'GAATTCATGGCTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGCCCAAAGTG
CCCAAGCACAGATCCAGTTGGTGCAGTCTG 3'

BKHC2 5'GCGCTGAGGTGAAGAAGCCTGGCGCTTCTGTGAAGGTGTCTTGCAAGGCTTCT
GGCTACATTCACATCTTACGCTATATCTTG 3'

BKHC3 5'GAATTGGGTGAGGCAGGCTCCTGGCCAGGGCCTGGAGTGGATGGGCTGGATAAAT
GGAAATGGAGATACAATTACGCCCAGAAG 3'

BKHC4 5'TTCCAGGGAAGGGTTACTATAACTGCTGATACTTCTACTTCTACTGCTTACATGG
AGCTGTCTTCTCTGAGGTCTGAGGATACT 3'

BKHC5 5'GCTGTTTACTACTGCGCTAGGGCTCCTGGCTACGGCTCTGATTATTGGGGACA
GGGAACACTGGTTACAGTTTCTTTCTGAATTC 3'

BKHC6 5'GAATTCAGAAGAAACTGTAACCAGTGTTCCCTGTCCCCAATAATCAGAGCCGTA
GCCAGGAGCC 3'

BKHC7 5' CTAGCGCAGTAGTAAACAGCAGTATCCTCAGACCTCAGAGAAGACAGCTCCAT
GTAAGCAGTAGAAGTAGAAGTATCAGCAGTT 3'

BKHC8 5'ATAGTAACCCTTCCCTGGAACTTCTGGGCGTAATTTGTATCTCCATTTCCATTT
ATCCAGCCCATCCACTCCAGGCCCTGGCCAG 3'

BKHC9 5'GAGCCTGCCTCACCCAATTCCAAGATATAGCGTAAGATGTGAATGTGTAGCCA
GAAGCCTTGCAAGACACCTTCACAGAAGCGCC 3'

BKHC10 5'AGGCTTCTTCACCTCAGCGCCAGACTGCACCAGCTGAACCTGTGCTTGGGCACT
TTGGGCAGCTGCCATCAGGAATAGCAAGGTCCACACCCAAGCCATGAATTC 3'

BKHCDR42 5'GCGCTGAGGTGAAGAAGCCTGGCGCTTCTGTGAAGGTGTCTTGCAAGGC
TTCTGGCTACACATTCACA 3'

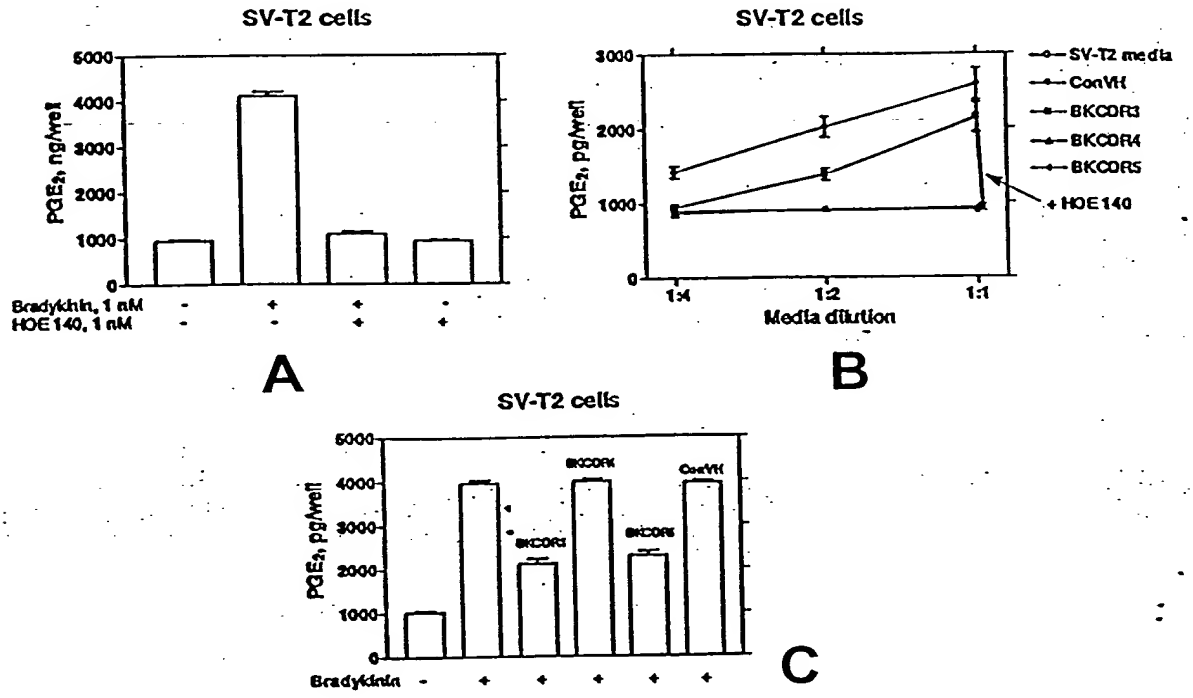
BKHDR43 5'CAGGTGGGTGAGGCAGGCTCCTGGCCAGGGCCTGGAGTGGATGGGCTGGAT
AAATGGAGATACAAATTACGCCCAGAAG 3'

BKHDR49 5'GAGCCTGCCTCACCCACCTGAAAGGAGAGAAGCCAGGTGTGAATGTGTA
GCCAGAAGCCTTGCAAGACACCTTCACAGAAGCGCC 3'

BKHDR53 5'GAATTGGGTGAGGCAGGCTCCTGGCCAGGGCCTGGAGTGGATGGGCTGGATA
AATGGAAGGCCTCCTGGCTTCTCTCCTTTCAGG 3'

BKHDR58 5'ATAGTAACCCTTCCCTGGAACCTGAAAGGAGAGAAGCCAGGAGGCCTTC
CATTTATCCAGCCCATCCACTCCAGGCCCTGGCCAG 3'

FIG. 6B


SV-T2 cells
PGE2, ng/well
5000
4000
3000
2000
1000
0
Bradykinin, 1 nM - + + -
HOE140, 1 nM - - + +
A
SV-T2 cells
PGE2, pg/well
3000
2000
1000
0
1:4
1:2
1:1
Media dilution
SV-T2 media
ConVH
BKCOR3
BKCOR4
BKCOR5
+ HOE140
B
SV-T2 cells
PGE2, pg/well
5000
4000
3000
2000
1000
0
BKCOR3
Bradykinin - + + + + +
C

FIGS. 7A-C

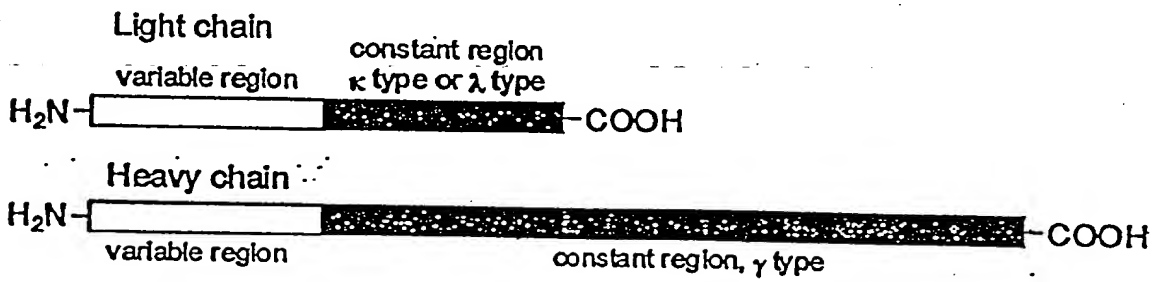

Light chain
variable region
constant region
κ type or λ type
H2N
COOH
Heavy chain
H2N
COOH
variable region
constant region, γ type

FIG. 1

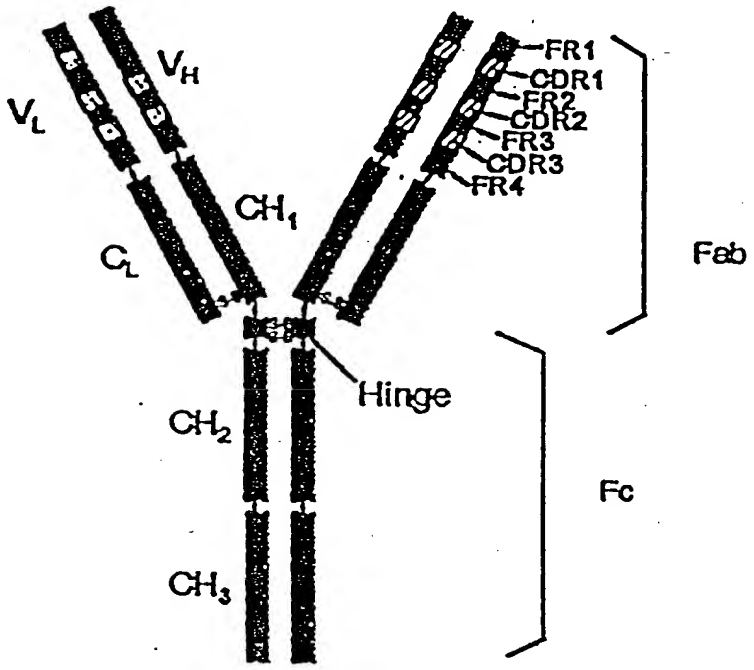


FIG. 2

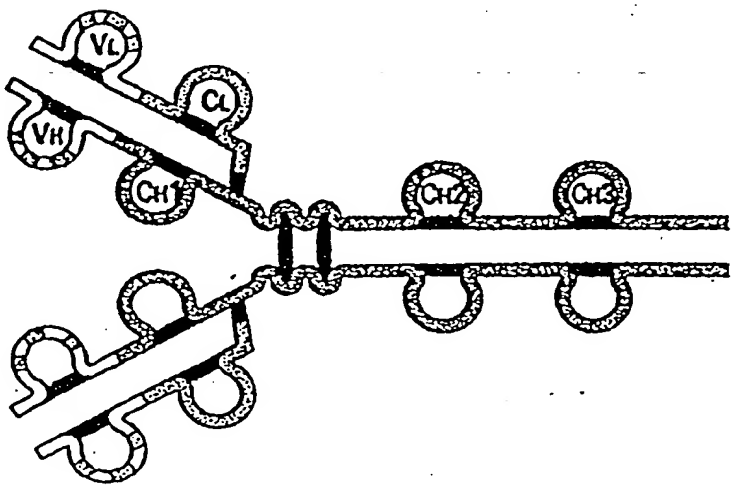


FIG. 3

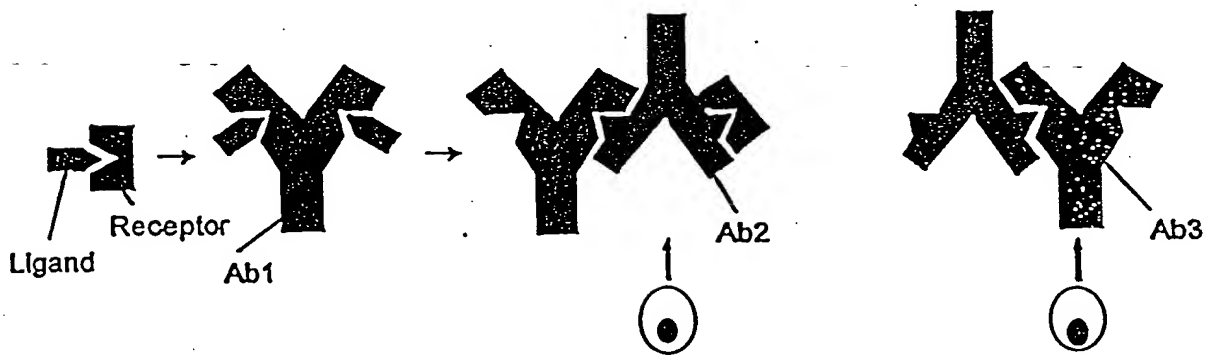


FIG. 4

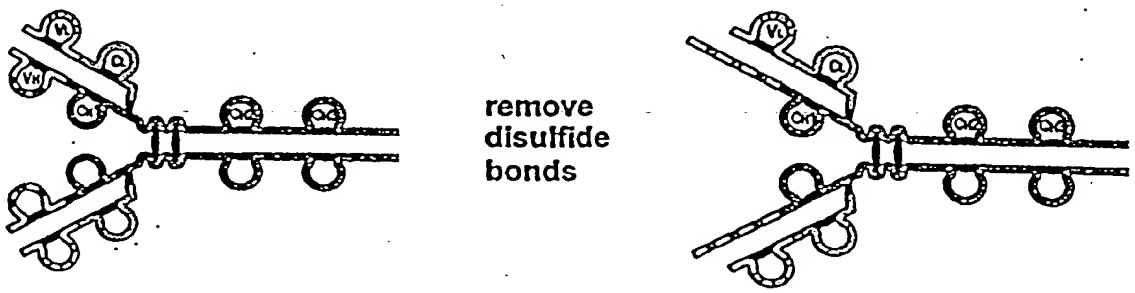


FIG. 5

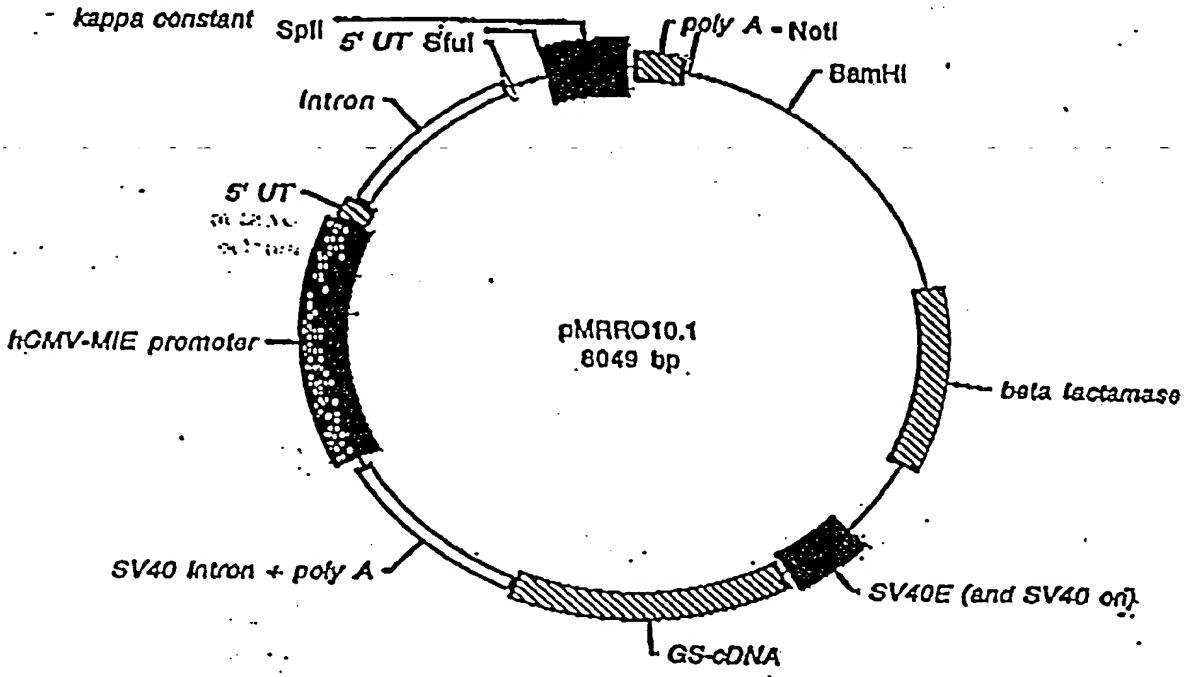

kappa constant
SpII
5' UT
SfuI
poly A - NotI
BamHI
Intron
5' UT
hCMV-MIE promoter
pMRRO10.1
8049 bp
beta lactamase
SV40 Intron + poly A
SV40E (and SV40 ori)
GS-cDNA

FIG. 6A

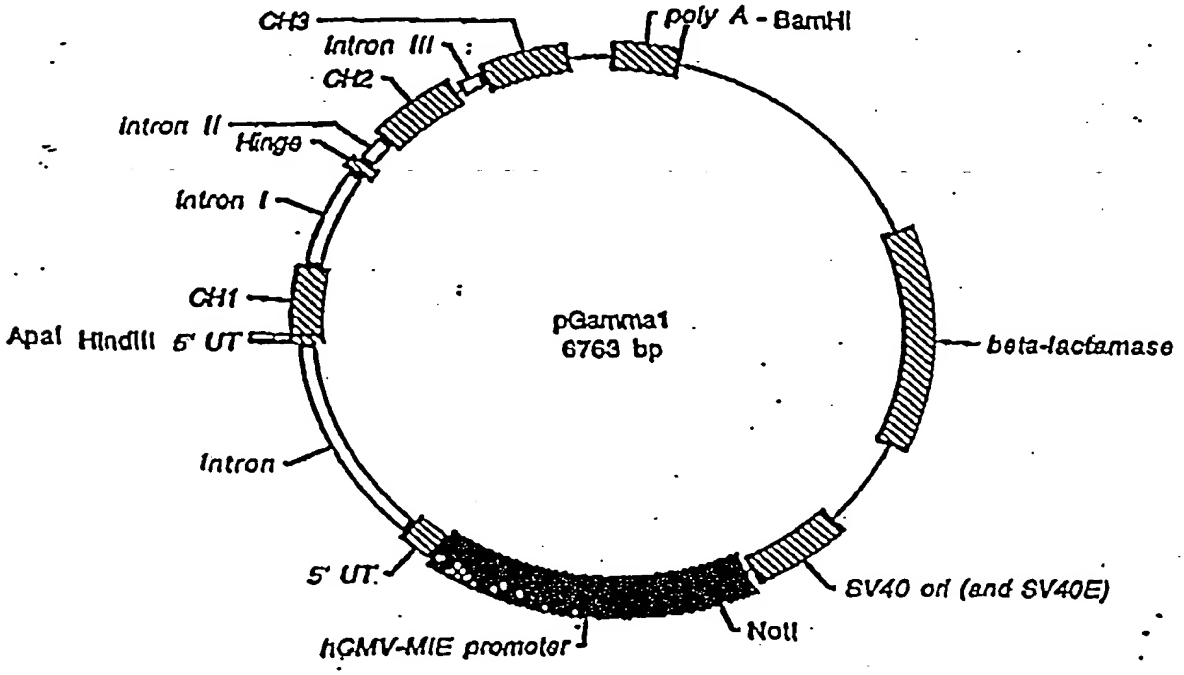

CH3
Intron III
CH2
Intron II
Hinge
Intron I
CH1
ApaI HindIII 5' UT
Intron
5' UT
hCMV-MIE promoter
NotI
SV40 ori (and SV40E)
beta-lactamase
poly A - BamHI
pGamma1
6763 bp

FIG. 6B

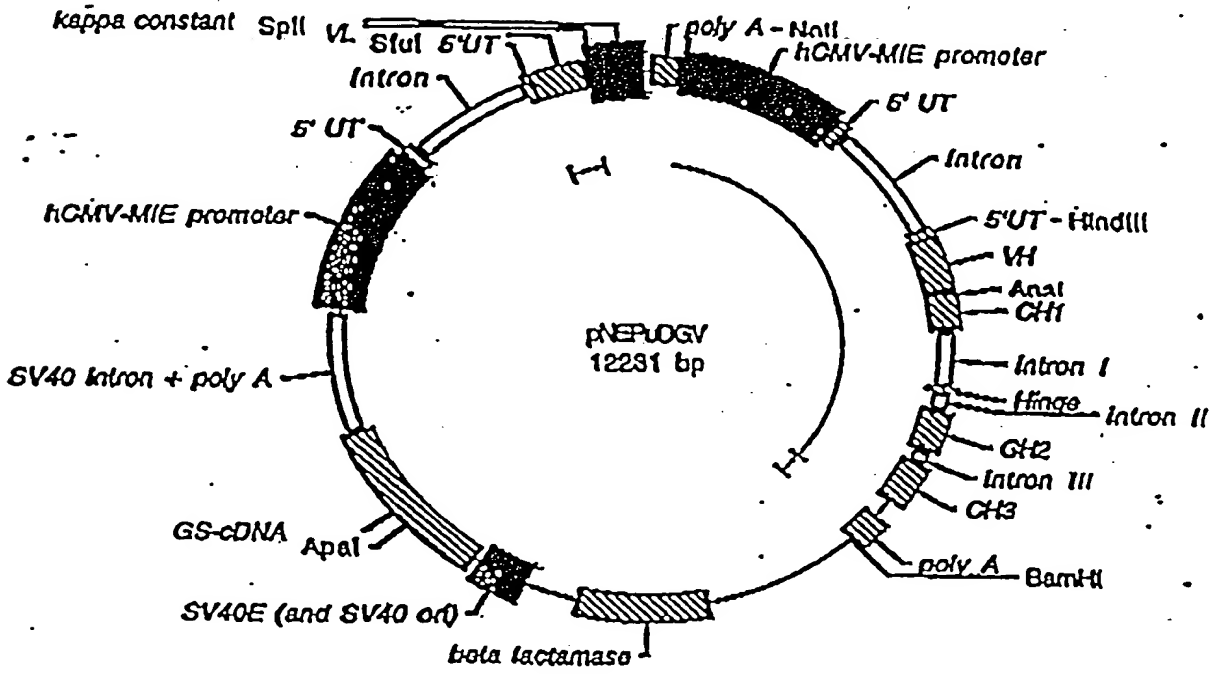

kappa constant
SpII
VL
SfuI 5'UT
intron
5' UT
hCMV-MIE promoter
SV40 intron + poly A
GS-cDNA
ApaI
SV40E (and SV40 ori)
beta lactamase
poly A - NotI
hCMV-MIE promoter
5' UT
intron
5'UT - HindIII
VH
AnaI
CH1
intron I
hinge
intron II
CH2
intron III
CH3
poly A
BamHI
pNEPUOGV
12231 bp

FIG. 6C

ConVL1

EcoR1
GAA TTC

6

-19 (Leader) -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
63

V_L:

1 10 20
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
Thr
GAT ATC CAA ATG ACA CAA AGT CCT AGT AGT TTG AGT GCT AGT GTG GGA GAT CGG GTG
ACA 123

21 30 40
Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys
Pro
ATC ACA TGT CGG GCT AGT CAA AGT ATC AGT AAC TGT TTG GCT TGG TAT CAA CAA AAG
CCT 183

41 50 60
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro
Ser
GGA AAG GCT CCT AAG TTG TTG ATC TAT GCT GCT AGT AGT TTG GAG AGT GGA GTG CCT
AGT 243

61 70 80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Arg Phe Thr Leu Thr Ile Ser Ser Leu Gln
Pro
CGG TTC AGT GGA AGT GGA AGT GGA ACA CGG TTC ACC TTG ACC ATC AGT AGT TTG CAA
CCT 303

81 90 100
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Leu Pro Trp Thr Phe Gly
Gln
GAG GAT TTC GCT ACC TAT TAT TGT CAA CAA TAT AAC AGT TTG CCT TGG ACC TTC GGA
CAA 363

101
Gly Thr Lys Val Glu Ile Lys
GGA ACC AAG GTG GAG ATC AAG GAA TTC 390
EcoRI

FIG. 7A

ConVH1

EcoR1
GAA TTC 6

-19 (Leader) -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala
Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC
CAA AGT GCC CAA GCA 63

V_L:
1 10 20
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro
Gly Ala Ser Val Lys Val
CAG GTT CAG CTG GTG CAG TCT GGC GCT GAG GTG AAG AAG CCT
GGC GCT TCT GTG AAG GTG 123

21 30 35A 35B
40
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Ala Ile
Ser Trp Asn Trp Val Arg Gln Ala
TCT TGC AAG GCT TCT GGC TAC ACA TTC ACA TCT TAC GCT ATA
TCT TGG AAT TGG GTG AGG CAG GCT 189

41 50 60
Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Gly Asn
Gly Asp Thr Asn Tyr Ala
CCT GGC CAG GGC CTG GAG TGG ATG GGC TGG ATA AAT GGA AAT
GGA GAT ACA AAT TAC GCC 249

61 70 80
Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser
Thr Ser Thr Ala Tyr Met
CAG AAG TTC CAG GGA AGG GTG ACT ATA ACT GCT GAT ACT TCT
ACT TCT ACT GCT TAC ATG 309

81 82A 82B 82C 90
100
Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr
Cys Ala Arg Ala Pro Gly Tyr Gly Ser
GAG CTG TCT TCT CTG AGG TCT GAG GAT ACT GCT GTT TAC TAC
TGC GCT AGG GCT CCT GGC TAC GGC TCT 378

101 110
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
GAT TAT TGG GGA CAG GGA ACA CTG GTT ACA GTT TCT TCT GAA TTC
423

FIG. 7B

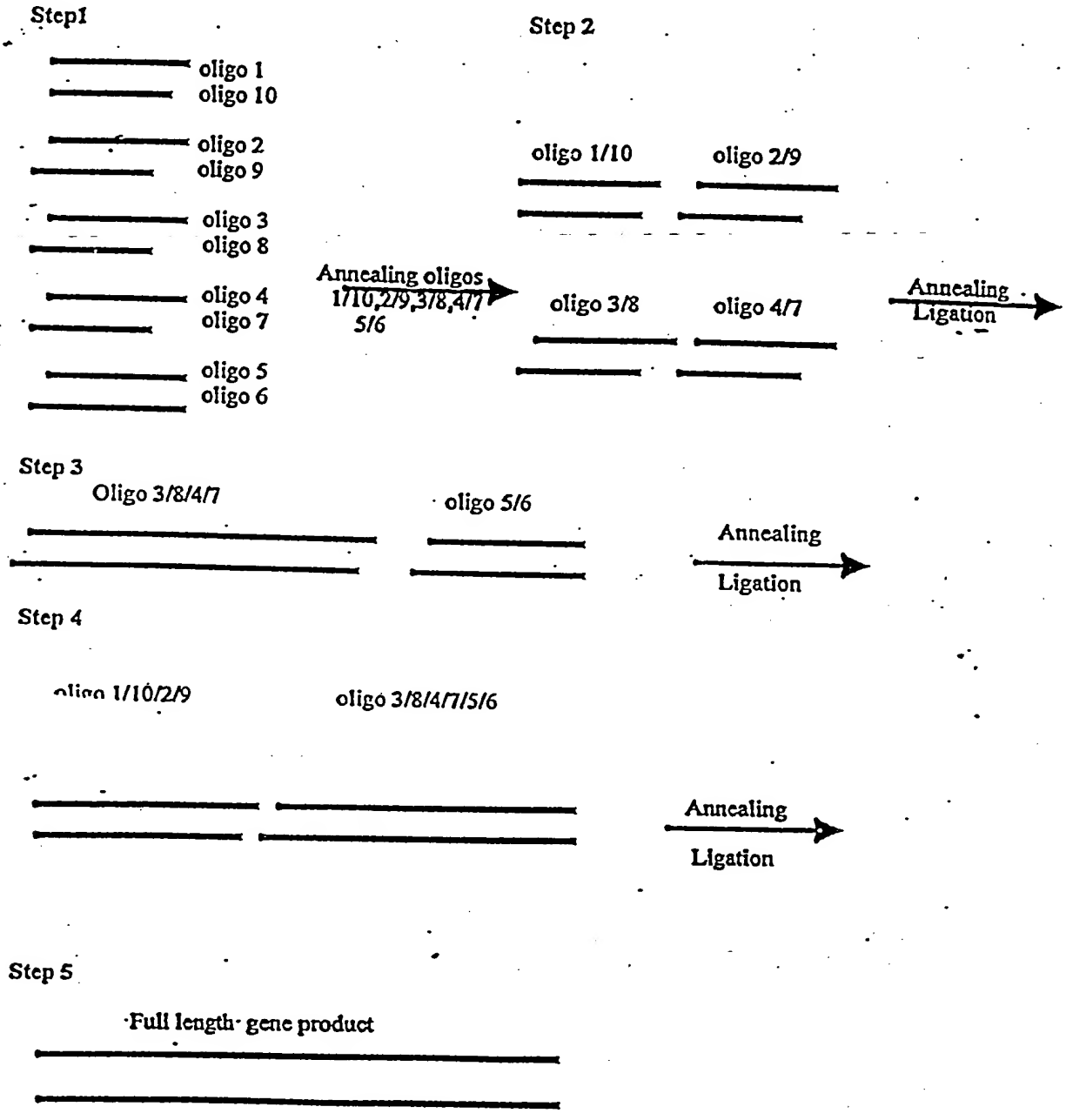

Step1
oligo 1
oligo 10
oligo 2
oligo 9
oligo 3
oligo 8
oligo 4
oligo 7
oligo 5
oligo 6
Annealing oligos
1/10,2/9,3/8,4/7
5/6
Step 2
oligo 1/10
oligo 2/9
oligo 3/8
oligo 4/7
Annealing
Ligation
Step 3
Oligo 3/8/4/7
oligo 5/6
Annealing
Ligation
Step 4
oligo 1/10/2/9
oligo 3/8/4/7/5/6
Annealing
Ligation
Step 5
Full length gene product

FIG. 8

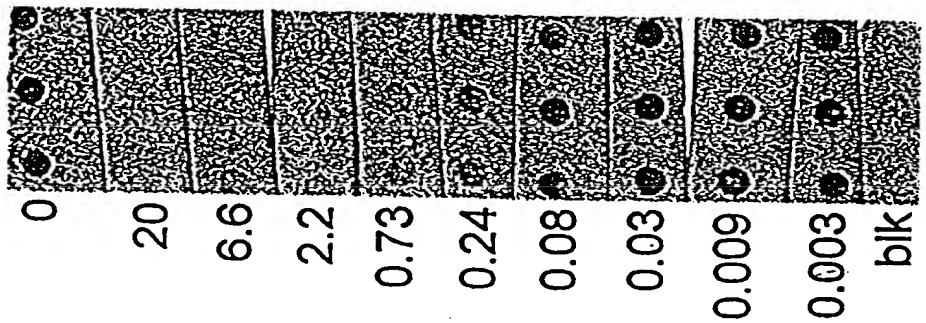
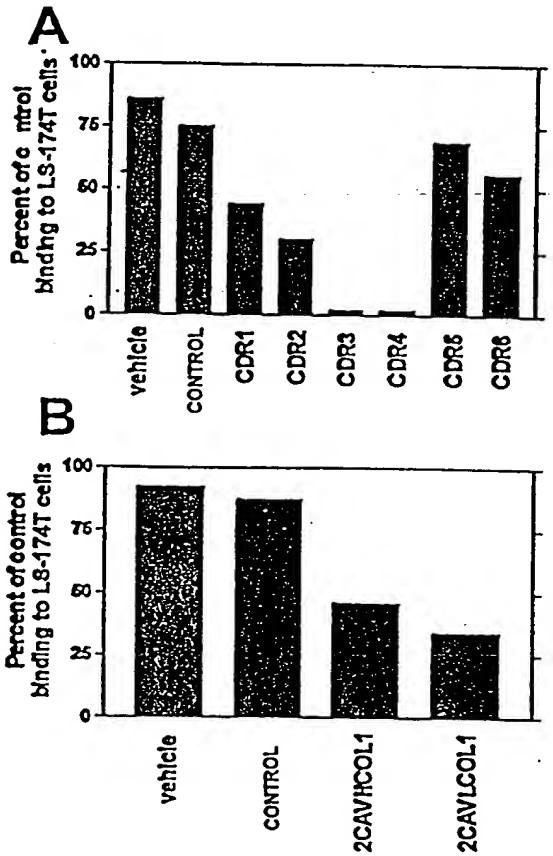
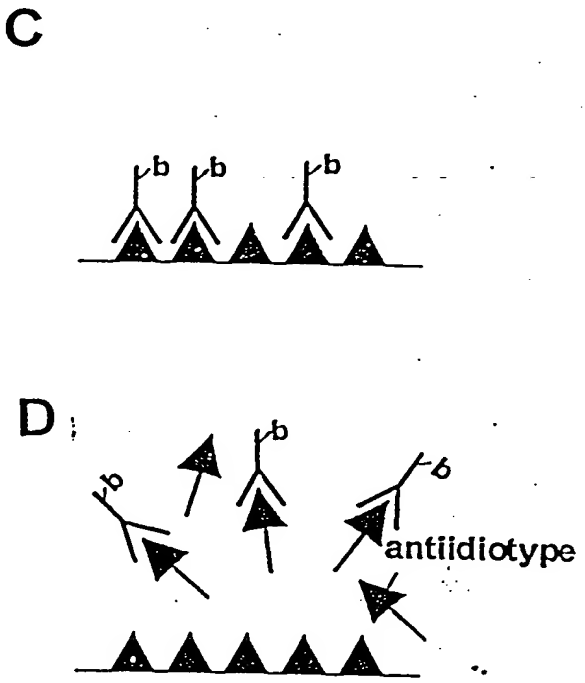


FIG. 9


A
Percent of control binding to LS-174T cells
100
75
50
25
0
vehicle
CONTROL
CDR1
CDR2
CDR3
CDR4
CDR5
CDR6
B
Percent of control binding to LS-174T cells
100
75
50
25
0
vehicle
CONTROL
2CAVHCOL1
2CAVLCOL1


C
b
b
b
D
b
b
b
antiidiotype

FIGS. 10A-D

cstacy
DSABL-1 GACATTGTGATGTCACAGTCTCCATCCTCCCTAGCTGTGTCAGTTGGAGAGAAGGTTACTATG 0.05
PAGE 63
DSABL-1c
GCAGCTCATAGTAACCTTCTCTCCAACTGACACAGCTAGGGAGGATGGAGACTGTGACATCACAATGTCTGC
TTGGGC 0.05 PAGE 78
MSAL-CDR1-1 ← GCT in MSALVAC-CDR1-1
AGCTGCGTCGGCAGCCTCCGAAGCAGCCCGCTCCAGAGCCCGCTGCTCCGATGGTACCAGCAGAAACCAG
GGCAGTCTCCTAAA 0.05 PAGE 84
MSAL-CDR1-1c
CTGCCCTGGTTTCTGCTGGTACCATCGGAGCAGCGGGCTCTGGAGCGGGCTGCTTCGGAGGCTGCCGAC
0.05 PAGE 69

HMVL1 GACATTGTGATGTCACAGTCTCCATCCTCCCTAGCTGTGTCAGTTGGAGAGAAGGTTACTATGAGCCCTAAGTCCAGT
HMVL2 CAGAGCCTTTTATATAGTAGCAATCAAAAGATCTACTTGGCCTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAA
HMVL3 CTGCTGATTTACTGGGCATCCACTAGGGAATCTGGGGTCCCTGATCGCTTCACAGGCGGTGGATCTGGG
HMVL5 GCACAGCAATATTATAGATATCCTCGGACGTTCGGTGGAGGCACCAAGCTGGAAATCAAACGGGAATTC
HMVL8 ACCGCCTGTGAAGCGATCAGGGATCCCAGATTCCCTAGTGGATGCCCAGTAAATCAGGAGTTTAGGAGA
HMVL9 CTGCCCTGGTTTCTGCTGGTACCAGGCCAAGTAGATCTTTTGATTGCTACTATATAAAAGGCTCTGACTGGACTT
HMVL10 AGCCCTCATAGTAACCTTCTCTCCAACTGACACAGCTAGCGACGATGGAGACTGTGACATCACAATGTCTCCTTGGGC
HMVL6 GAATTCCCGTTTGATTTCCAGCTTGGTGCCTCCACCGAACGTCCGAGGATATCTATAATATTGCTGTGCGTAATAAAC

HMVL4
IAG AGA TTT CAG TCT CAG CAT CAG CAG TGT GAA GGC TGA AGA CGT GGC
AGT TTA TTA C

HMVL7
TG GCA GGT CTT CAG CGT TCA CAC TGC TGA TGG TGA GAG TGA AAT CTG
TCC CAG ATC C

FIG. 11

A

MSA-63 epitope DNA
GTC GGC AGC CTC CGA AGC AGC CCG CTC CAG AGC CCG CTG CTC CGA CCG CTC GTC
CAG AGC AGC CTC TGC TTG CTG TTC CTC TTG CTG CGA TAC AGC TGC GGC GAC GGC AGC
TGC AGC CGA CGA TAC TGC GAC TTG ACG GTG TGC ACG CGA ATG TAC TTG CTG CTG
CGA TTC ACG GAC GCG CCG CTC CCG CAG ACG TGC TGC GTC TTG AGC

B

MSA-63 protein sequence (Start residue 143 end residue 233)
Gln Pro Ser Glu Ala Ser Ser Gly Glu Val Ser Gly Asp Glu Ala Gly Glu Gln Val Ser Ser Glu Thr Asn Asp
Lys Glu Asn Asp Ala Met Ser Thr Pro Leu Pro Ser Thr Ser Ala Ala Ile Thr Leu Asn Cys His Thr Cys Ala
Tyr Met Asn Asp Asp Ala Lys Cys Leu Arg Gly Glu Gly Val Cys Thr Thr Gln Asn Ser

C

MSA-63 oligo

MSA1
GTC GGC AGC CTC CGA AGC AGC CCG CTC CAG AGC CCG CTG CTC CGA

MSA2
AGC CCG CTG CTC CGA CCG CTC GTC CAG AGC AGC CTC TGC TTG CTG

MSA3
AGC CTC TGC TTG CTG TTC CTC TTG CTG CGA TAC AGC TGC GGC GAC

MSA4
TAC AGC TGC GGC GAC GGC AGC TGC AGC CGA CGA TAC TGC GAC TTG

MSA5
CGA TAC TGC GAC TTG ACG GTG TGC ACG CGA ATG TAC TTG CTG CTG

MSA6
ATG TAC TTG CTG CTG CGA TTC ACG GAC GCG CCG CTC CCG CAG ACG

MSA7
CGA TTC ACG GAC GCG CCG CTC CCG CAG ACG TGC TGC GTC TTG AGC

FIGS. 12A-C

A

SP-10 Epitope
GAA TTC CAG CCT TCA GGT GAA CAT GGC TCC GGT GAA CAG CCT TCT GGT GAG CAG
GCC TCG GGT GAA CAG CCT TCA GGT GAG CAC GCT TCA GGG GAA CAG GCT TCA GGT
GCA CCA ATT TCA AGC ACA TCT ACA GGC ACA ATA TTA AAT TGC TAC ACA TGT GCT TAT
ATG AAT GAT CAA GGA AAA TGT CTT CGT GGA GAG GGA ACC TGC ATC ACT CAG AAT TC

B

SP-10 protein sequence
Gln Pro Ser Gly Glu His Gly Glu Gln Pro Ser Gly Glu Gln Ala Ser Gly Glu Gln Pro Ser gly Glu His Ala
Ser Gly Glu Gln Ala Ser Gly Ala Gln Ile Ser Ser Thr Ser Thr Gly Thr Ile Leu Asn Cys Tyr Thr Cys Ala
Tyr Met Asn Asp Gln Gly Lys Cys Leu Arg Gly Glu Gly Thr Cys Ile Thr Gln Asn

C

Oligo SP1:
GAA TTC CAG CCT TCA GGT GAA CAT GGC TCC GGT GAA CAG CCT TCT GGT GAG CAG
GCC TCG GGT GAA CAG CCT TAG

Oligo SP2:
GTG AGC ACG CTT CAG GGG AAC AGG CTT CAG GTG CAC CAA TTT CAA GCA CAT CTA
CAG GCA CAA TAT TAA ATT GCT

Oligo SP3:
ACA CAT GTG CTT ATA TGA ATG ATC AAG GAA AAT GTC TTC GTG GAG AGG GAA CCT
GCA TCA CTC AGA ATT C

Oligo SP3a(3Cys-> Ala):
ACA CAG CAG CTT ATA TGA ATG ATC AAG GAA AAG CAC TTC GTG GAG AGG GAA
CCG CAA TCA CTC AGA ATT C

Oligo SP4:
GAA TTC TGA GTG ATG CAG GTT CCC TCT CCA CGA AGA CAT TTT CCT TGA TCA TTC ATA
TAA GCA CAT GTG TAG CAA TTT A

Oligo SP4a (3Cys->Ala):
GAA TTC TGA GTG ATT GCG GTT CCC TCT CCA CGA AGT GCT TTT CCT TGA TCA TTC ATA
TAA GCT GCT GTG TAG CAA TTT A

Oligo SP5:
ATA TTG TGC CTG TAG ATG TGC TTG AAA TTG GTG CAC CTG AAG CCT GTT CCC CTG AAG
CGT GCT CAC CTG AAG GCT

Oligo SP6:
GTT CTC CCG AGG CCT GCT CAC CAG AAG GCT GTT CAC CGG AGC CAT GTT CAC CTG
AAG GCT GGA ATT C

FIGS. 13A-C

LDH-C_4 Epitope:

Oligo LDH1:
TCG TGC CAG TTC CTC GTC GAC TAG CTC TTC GAC TAG CTC CTG CTG CTC TTG TCG GTC ACG GAA TTC

Oligo LDH2:
GAA TTC CGT GAC CGA CAA GAG CAG CAG GAG CTA GTC GAA GAG CTA GTC GAC GAG GAA CTG GCA CGA CGG GTT CGT

FIG. 14

Leader:

```
-19                                                                  -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
```

V_L:

Ala GCT in vaccine

```
1                                   10                                  20
Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala Val Ser Val Gly Glu Lys Val Thr
GAC ATT GTG ATG TCA CAG TCT CCA TCC TCC CTA GCT GTG TCA GTT GGA GAG AAG GTT ACT

21                        27 A  B  C  D  E  F          30                  40
Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser Ser Asn Gln Lys Ile Tyr Leu Ala Trp Tyr Gln Gln Lys Pro
ATG AGC TGC AAG TCC AGT CAG AGC CTT TTA TAT AGT AGC AAT CAA AAG ATC TAC TTG GCC TGG TAC CAG CAG AAA CCA
                                  CDR1
41                                  50                                  60
Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Asp
GGG CAG TCT CCT AAA CTG CTG ATT TAC TGG GCA TCC ACT AGG GAA TCT GGG GTC CCT GAT
                                  CDR2
61                                  70                                  80
Arg Phe Thr Gly Gly Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Lys Ala
CGC TTC ACA GGC GGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGT GTG AAG GCT

81                                  90                                 100
Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr Arg Tyr Pro Arg Thr Phe Gly Gly
GAA GAC CTG GCA GTT TAT TAC TGT CAG CAA TAT TAT AGA TAT CCT CGG ACG TTC GGT GGA
                                  CDR3
101
Gly Thr Lys Leu Glu Ile Lys Arg
GGC ACC AAG CTG GAA ATC AAA CGG
```

FIG. 15

2CAVHCOL1

VHC1 5'GAATTCATGGCTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGCCCAAAGTGCCC
AAGCACAGATCCAGTTGGTGCA 3'

VHC2 5'GTCTGGACCTGAGCTGAAGAAGCCTGGAGAGACAGTCAAGATCTCCGCTAAGGCTTC
TGGGTATACCTTCACAAACTAG 3'

VHC3 5'GAATGAACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGGGCTGGAT
AAACACCTACACTGGAGAGCCAACA 3'

VHC4 5'TATGCTGATGACTTCAAGGGACGGTTTGCCTTCTCTTTGGAAACCTCTGCCAGCACT
GCCTATTTGCAGATCAACACCT 3'

VHC5 5'CAAAAATGAGGACACGGCTACATATTTCGCTGCAAGAGCCTACTATGGTAAATAC
TTTGACTACGAATTC 3'

VHC6 5'GAATTCGTAGTCAAAGTATTTACCATAGTAGGCTCTTGCAGCAAATATG 3'

VHC7 5'TAGCCGTGTCCTCATTTTTGAGGTTGTTGATCTGCAAATAGGCAGTGCTGGCAGA
GGTTTCCAAAGAGAAGGCAAACCGT 3'

VHC8 5'CCCTTGAAGTCATCAGCATATGTTGGCTCTCCAGTGTAGGTGTTTATCCAGCCCAT
CCACTTTAAACCCTTTCCTGGAGC 3,

VHC9 5'CTGCTTCACCCAGTTCATTCCATAGTTTGTGAAGGTATACCCAGAAGCCTTAGCGG
AGATCTTGACTGTCTCTCCAGGCT 3'

VHC10 5'TCTTCAGCTCAGGTCCAGACTGCACCAACTGGATCTGTGCTTGGGCACTTTG GGC
AGCTGCCATCAGGAATAGCAAGGTCCACACCCAAGCCATGAATTC 3'

FIG. 16A

2CAVLCOL1

VLC1 5'AGTATTGTGATGACCCAGACTCCCAAATTCCTGCTTGTATCAGCAGGAGACAGGGTT
ACCATA 3'

VLC2 5'ACCTGCAAGGCCAGTCAGAGTGTGAGTAATGATGTAGCTTGGTACCAACAGAAAACC
AGGGCAG 3'

VLC3 5'TCTCCTAAACTGCTGATATACTATGCATCCAATCGCTACACTGGAGTCCCTGATCGCT
TCACTGGCAGT 3'

VLC4 5'GGATATGGGACGGATTTCACTTTCACCATCAGCACTGTGCAGGCTGAAGACCTGGCA
GTTTAT 3'

VLC5 5'TTCTGYCAGCAGGATTATAGCTCTCCGCTCACGTTCGGTGCTGGGACCAAGCTGGAG
CTGAAAGAATTC 3'

VLC6 5'GAATTCTTTCAGCTCCAGCTTGGTCCCAGCACCGAACGTGAGCGGAGAGCTATAATC
CTGCTGACAGAAATAAACTGC 3'

VLC7 5'CAGGTCTTCAGCCTGCACAGTGCTGATGGTGAAAGTGAAATCCGTCCCATATCCA
CTGCCAGT 3'

VLC8 5'GAAGCGATCAGGGACTCCAGTGTAGCGATTGGATGCATAGTATATCAGCAGTTTAG
GAGACTGCCCTGG 3'

VLC9 5'TTTCTGTTGGTACCAAGCTACATCATTACTCACACTCTGACTGGCCTTGCAGGTTA
TGGTAAC 3'

VLC10 5'CCTGTCTCCTGCTGATACAAGCAGGAATTTGGGAGTCTGGGTCATCACAATACTT
GCTTGGGC 3'

VLC11 5'TTCGCTCAGCAGGATTATAGCTCTCCGCTCACGTTCGGTGCTGGGACCAAGCTGG
AGCTGAAAGAATC 3'

VLC12 5'GAATTCTTTCAGCTCCAGCTTGGTCCCAGCACCGAACGTGAGCGGAGAGCTATAA
TCCTGCTGAGCGAAATAAACTGC 3'

FIG. 16B

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(54) Title: CONTRACEPTIVE ANTIBODY VACCINES

(57) Abstract: The invention provides an antibody contraceptive vaccine comprising an antibody that has at least one CDR containing a portion of an antigen of a cell or protein associated with reproductive function and which antibody has an enhanced ability to elicit an anti-idiotype response, for example, by substituting one or more variable region cysteine residues that form intrachain disulfide bonds with an amino acid residue that does not have a sulfhydryl group, such that the intrachain disulfide bond does not form. The invention further provides methods of contraception using the antibody contraceptive vaccines of the invention.

CONTRACEPTIVE ANTIBODY VACCINES

1. FIELD OF THE INVENTION

The present invention relates to modified antibodies, and vaccine compositions thereof, that have one or more complementary determining regions that contain portions of sperm antigens, in which modified antibodies one or more variable region cysteine residues that form intrachain disulfide bonds have been replaced with amino acid residues that do not contain a sulfhydryl group and, therefore, do not form disulfide bonds. The present invention also relates to use of the vaccine compositions of the invention as a contraceptive.

2. BACKGROUND OF THE INVENTION

2.1. IMMUNOGLOBULIN STRUCTURE

The basic unit of immunoglobulin structure is a complex of four polypeptides -- two identical low molecular weight or "light" chains and two identical high molecular weight or "heavy" chains, linked together by both noncovalent associations and by disulfide bonds. Each light and heavy chain of an antibody has a variable region at its amino terminus and a constant domain at its carboxyl terminus (Figure 1). The variable regions are distinct for each antibody and contain the antibody antigen binding site. Each variable domain is comprised of four relatively conserved framework regions and three regions of sequence hypervariability termed complementarity determining regions or CDRs (Figure 2). For the most part, it is the CDRs that form the antigen binding site and confer antigen specificity. The constant regions are more highly conserved than the variable domains, with slight variations due to haplotypic differences.

Based on their amino acid sequences, light chains are classified as either kappa or lambda. The constant region heavy chains are composed of multiple domains (CH1, CH2, CH3...CHx), the number depending upon the particular antibody class. The CH1 region is separated from the CH2 region by a hinge region which allows flexibility in the antibody. The variable region of each light chain aligns with the variable region of each heavy chain, and the constant region of each light chain aligns with the first constant region of each heavy chain. The CH2-CHx domains of the constant region of a heavy chain form an "Fc region" which is responsible for the effector functions of the immunoglobulin molecule, such as complement binding and binding to the Fc receptors expressed by lymphocytes, granulocytes, monocyte lineage cells, killer cells, mast cells and other immune effector cells.

As seen in Figure 3. the light and heavy chains of an IgG molecule form the variable region domain and the constant region domain. Each domain is composed of a sandwich of two parallel extended protein layers of about 100 amino acids in length which are connected by a single disulfide bond (See Roitt et al., Immunology, 3rd Edition, London: Mosby, 1993, p4.4 (Figure 3)). Each of the two extended protein layers of the domain, in turn, contains two "anti-parallel" adjacent strands which adopt a beta-sheet conformation. (See, *e.g.*, Stryer, 1975, Biochemistry, WH Freeman and Co., p. 950). Each of the domains has a similar three-dimensional structure based on the immunoglobulin fold.

2.2. IMMUNOTHERAPY AND ANTI-IDIOTYPE ANTIBODIES

In modern medicine, immunotherapy or vaccination has virtually eradicated diseases such as polio, tetanus, tuberculosis, chicken pox, measles, hepatitis, etc. The approach using vaccinations has exploited the ability of the immune system to prevent infectious diseases.

Use of immunotherapy has also been explored for cancer therapy. The era of tumor immunology began with experiments by Prehn and Main, who showed that antigens on the methylcholanthrene (MCA)-induced sarcomas were tumor specific in that transplantation assays could not detect these antigens in normal tissue of the mice (Prehn et al., 1957, J. Natl. Cancer Inst. 18:79-778). This notion was confirmed by further experiments demonstrating that tumor specific resistance against MCA-induced tumors could be elicited in the autochthonous host, that is, the mouse in which the tumor originated (Klein et al., 1990, Cancer Res. 20:151-1572).

There are many reasons why immunotherapy is desired for use in cancer patients. First, if cancer patients are immunosuppressed in surgery, with anesthesia and subsequent chemotherapy, it may worsen the immunosuppression, then with appropriate immunotherapy in the preoperative period, this immunosuppression may be prevented or reversed. This could lead to fewer infectious complications and accelerated wound healing. Second, tumor bulk is minimal following surgery and immunotherapy is most likely to be effective in this situation. A third reason is the possibility that tumor cells are shed into the circulation at surgery and effective immunotherapy applied at this time can eliminate these cells.

There are two types of immunotherapy, the "active immunotherapy" and the "passive immunotherapy". In "active immunotherapy", an antigen is administered in the form of a vaccine, to a patient, so as to elicit a protective immune response. "Passive immunotherapy" involves the administration of antibodies to a patient without eliciting a concommitant immune response. When a specific antibody from one animal is injected as

an immunogen into a suitable second animal, the injected antibody will elicit an immune response. Antibody therapy is conventionally characterized as passive since the patient is not the source of the antibodies. However, the term passive is misleading because the patient can produce anti-idiotypic secondary antibodies which in turn provoke an immune response which is cross-reactive with the original antigen. Immunotherapy where the patient generates secondary antibodies is often more therapeutically effective than passive immunotherapy because the patient's own immune system continues to fight the cells bearing the particular antigen well after the initial infusion of antibody.

In an anti-idiotype response, antibodies produced initially during an immune response or introduced into an organism will carry unique new epitopes to which the organism is not tolerant, and therefore will elicit production of secondary antibodies (termed "Ab2"), some of which are directed against the idiotype (*i.e.*, the antigen binding site) of the primary antibody (termed "Ab1"), *i.e.*, the antibody that was initially produced or introduced exogenously. These secondary antibodies or Ab2 likewise will have an idiotype, which will induce production of tertiary antibodies (termed "Ab3"), some of which will recognize the antigen binding site of Ab2, and so forth. This is known as the "network" theory. Some of the secondary antibodies will have a binding site which is an analog of the original antigen, and thus will reproduce the "internal image" of the original antigen. And, the tertiary or Ab3 antibodies that recognize this antigen binding site of the Ab2 antibody will also recognize the original antigen (Figure 4).

Therefore, anti-idiotypic antibodies have binding sites that are similar in conformation and charge to the antigen, and can elicit the same or greater response than that of the cancer antigen itself. Administration of an exogenous antibody that can elicit a strong anti-idiotypic response can thus serve as an effective vaccine, by maintaining a constant immune response.

To date, anti-idiotypic vaccines have comprised murine antibodies because the anti-idiotypic response occurs as part of the typical human anti-mouse antibody (HAMA) response. A strong anti-idiotypic cascade has been observed when Ab1 has been structurally damaged (Madiyalakan et al., 1995, *Hybridoma* 14:199-203), rendering the antibody more foreign. There has been direct administration to the subject of exogenously produced anti-idiotype antibodies that are raised against the idiotype of an anti-tumor antibody (U.S. Patent No. 4,918,14). After administration, the subject's body will produce anti-antibodies which not only recognize these anti-idiotype antibodies, but also recognize the original tumor epitope, thereby directing complement activation and other immune system responses to a foreign entity to attack the tumor cell that expresses the tumor epitope.

However, while anti-idiotypic vaccines are desirable targets and several have been identified, the ability to deliver antibodies that reproducibly cause the generation of such an anti-idiotypic response is not currently possible. (Foon et al., 1995, *J. Clin. Invest.* 9:334-342; Madiyalakan et al., 1995, *Hybridoma* 14:199-203). One of the reasons for the failure to generate an anti-idiotypic response is that, Ab1, while exogenous, is still very similar to "self", as all antibodies have very similar structures, and anti-idiotypic responses to self molecules tend to be very limited. Thus, there is a need in the art for methods of reliably generating an anti-idiotype response to a specific antibody.

2.3. CONTRACEPTIVE METHODS

A variety of contraceptive methods are currently available. Such methods include barrier methods such as condoms or diaphragms, or use of spermicidal agents such as non-oxynol-9, hormone therapies such as birth control pills or implants, and other methods such as intrauterine devices. All of these methods pose problems as convenient and effective methods of preventing conception. Some methods are inconvenient or ineffective, some pose health risks, while others are costly. Accordingly, there is a need in the art for a safe, inexpensive, and convenient method of contraception.

3. SUMMARY OF THE INVENTION

The present invention is based upon the realization of the present inventors that an antibody in which one or more variable region cysteine residues that form one or more intrachain disulfide bonds have been replaced with amino acid residues that do not contain sulfhydryl groups, such that the particular disulfide bonds do not form, elicit a much stronger anti-idiotype response than an antibody in which the variable region disulfide bonds are intact. Additionally, the present inventors have realized that portions of antigens of proteins or reproductive cells, particularly sperm antigens, can be inserted into or used to replace portions of one or more complementarity determining regions, such that the modified antibody can be used as a vaccine to generate anti-idiotype antibodies that recognize the particular antigen.

Accordingly, the present invention provides modified immunoglobulin molecules or antibodies (and functionally active fragments, derivatives and analogs thereof), and vaccine compositions containing these immunoglobulin molecules, wherein the variable region of the immunoglobulin is subject to decreased conformational constraints, such as, but not limited to, by breaking one or more intrachain or interchain disulfide bonds. Specifically, the invention provides modified immunoglobulins that comprise a variable region and are identical, except for one or more amino acid substitutions in said variable region, to a

second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding (*i.e.*, specific binding of the immunoglobulin to its antigen as determined by any method known in the art for determining antibody-antigen binding, which excludes non-specific binding but not necessarily cross-reactivity with other antigens) an antigen or having a CDR that contains a portion of an antigen, said one or more amino acid substitutions being the substitution of one or more amino acid residues that do not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule. In preferred embodiments, the second immunoglobulin molecule contains a CDR that contains a portion of an antigen of a cell or protein involved in reproductive function, preferably sperm antigens, more preferably the sperm antigens SP-10, LDH-C_4, or MSA-63.

The invention further provides methods of eliciting an anti-idiotype response in a subject by administering the modified immunoglobulins of the invention. In particular, the modified immunoglobulins of the invention can be used as contraceptives, either in males or, preferably in females, specifically by administering an immunoglobulin molecule of the invention, which immunoglobulin molecule was derived (*i.e.*, by modification according to the invention to replace one or more variable region cysteine residues that form an intrachain disulfide bond with an amino acid residue that does not contain a sulfhydryl group) from an immunoglobulin molecule that contains a CDR that contains a portion of an antigen of a protein or cell associated with reproductive function, preferably a sperm antigen.

The invention also provides methods of producing the modified immunoglobulin molecules of the invention and vaccine compositions containing the modified immunoglobulin molecules of the invention.

4. DESCRIPTION OF FIGURES

Figure 1. A schematic diagram showing the structure of the light and heavy chain of an immunoglobulin molecule, each chain consisting of a variable region positioned at the amino terminal region (H_2N-) and a constant region positioned at a carboxyl terminal region (-COOH).

Figure 2. A schematic diagram of an IgG showing the four framework regions (FR1, FR2, FR3 and FR4) and three complementarity determining regions (CDR1, CDR2 and CDR3) in the variable regions of the light and heavy chains (labeled as V_L and V_H, respectively). The constant region domains are indicated as C_L for the light chain constant domain and CH_1, CH_2 and CH_3 for the three domains of the heavy chain constant region. Fab indicates the portion of the antibody fragment which includes the variable region

domains of both light and heavy chains and the C_L and CH_1 domains. Fc indicates the constant region fragment containing the CH_2 and CH_3 domains.

Figure 3. A schematic diagram of an antibody structure as shown in Figure 2, but drawn to emphasize that each domain (the loop structures labeled as V_L, V_H, C_L, CH_1, CH_2, and CH_3, respectively) is structurally defined by a disulfide bond (indicated with darkest lines) that maintains the three-dimensional structure (Roitt et al., *Immunology*, Second Edition, London: Gower Medical Publishing, 1989, p 5.3).

Figure 4. A schematic diagram showing the development of internal image bearing anti-idiotype antibodies (Ab2) and anti-anti-idiotype antibodies (Ab3) from idiotype antibodies (Ab1) directed against an antigen of a tumor cell in an antiidiotypic cascade.

Figure 5. Modification of the variable region of an immunoglobulin by replacing cysteine residues in the variable regions with alanine residues to remove an intrachain disulfide bond. CH1, CH2 and CH3 are constant regions. V_H is the heavy chain variable region and V_L is the light chain variable region.

Figures 6A-C. (A). The structure of the expression vector pMRRO10.1, which contains a human kappa light chain constant region sequence. (B). The structure of the expression vector pGamma1 that contains a sequence encoding a human IgG1 constant region (CH1, CH2, CH3) heavy chain and hinge region sequences. (C) The structure of the expression vector pNEPuDGV which contains a sequence encoding the kappa constant domain of the light chain and the constant domain and hinge region of the heavy chain. For all three vectors see Bebbington et al., 1991, *Methods in Enzymology* 2:136-145.

Figures 7A and B. (A) The amino acid sequence and corresponding nucleotide sequence for the consensus light chain variable region ConVL1. (B) The amino acid and corresponding nucleotide sequences for the consensus heavy chain variable region ConVH1.

Figure 8. A schematic diagram of the general steps that were followed for the assembly of an engineered gene encoding the synthetic modified antibody specific to human colon cancer antigen.

Figure 9. Dot blot showing the result of an assay for the competition of binding of the antibody derived from mAB31.1, but not having the cysteine to alanine changes with the same antibody which is biotin labeled to an antigen preparation derived from LS-174 T-cells. The concentration of the unlabeled antibody is indicated as nM unlabeled antibody. The "blk" lane has no antigen.

Figures 10A-D. (A)Results of competition binding assay of the biotin-labeled anti-colon carcinoma cell antibody to LS-174T cells in the presence of antisera from mice vaccinated with vehicle alone, control antibody that binds the colon carcinoma cell antibody

but has not been modified, and peptides CDR1, CDR2, CDR3, CDR4, CDR5, and CDR6, having the CDR sequences containing the bradykinin receptor binding site expressed as percent of control binding to LS-174T cells. (B). Results of competition binding assays of the biotin-labeled anti-colon carcinoma cell antibody to LS-174T cells in the presence of antisera from mice vaccinated with vehicle alone, control antibody that binds the colon carcinoma cell antibody, but has not been modified, 2CAVHCOL1, and 2CAVLCOL1. (C) Diagram showing the binding of a biotin-labeled (indicated by the "b") antibody (inverted Y) to antigen (solid triangles). (D) Diagram showing the inhibition of binding of the biotin-labeled (indicated by the "b") antibody (inverted Y) by anti-idiotype antibodies (solid arrows) to antigen (solid triangles).

Figure 11. Nucleotide sequences of the oligonucleotides used to construct the MSA1 and MSALVAC-1 variable regions.

Figures 12A-C. (A) Nucleotide sequence for the MSA-63 epitope. (B) Amino acid sequence of the MSA-63 epitope encoded by the nucleotide sequence of Figure 12A. (C) MSA-63 oligonucleotides used to construct a modified variable region. Each oligo overlaps for five codons and transitions the entire sequence of Figure 12A.

Figures 13 A-C. (A) Nucleotide sequence for the SP-10 epitope. (B) Amino acid sequence of the SP-10 epitope encoded by the nucleotide sequence of Figure 13A. (C) Oligonucleotides of Sp-10 used to construct a modified variable region. SP3a and SP4a are modified to change the codons encoding certain cysteine residues to codons encoding alanine residues.

Figure 14. Oligonucleotides of LDH-C4 epitope sequence for construction of a modified variable region gene containing a LDH-C_4.

Figure 15. Nucleotide and amino acid sequence of the consensus contraceptive light chain variable region.

Figure 16 A-B. (A) Sequences of oligos used in the construction of 2CAVHCOL1. (B) Sequences of oligos used in the construction of 2CAVLCOL1.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention provides modified immunoglobulins (particularly antibodies and functionally active fragments, derivatives, and analogs thereof) that can be used as contraceptive vaccines. Specifically, these antibodies have one or more complementarity determining regions (CDRs) that contain a portion of an antigen of a cell or protein involved in reproductive functio, preferably a sperm antigen. In addition, these antibodies have been engineered to elicit a stronger immune response, particularly a stronger anti-idiotypic response, than the corresponding unmodified immunoglobulins. In particular, the

modified immunoglobulins of the invention are immunoglobulins that are modified to decrease the conformational constraints on one variable region of the immunoglobulin molecule, preferably, such that at least one of the cysteines that participates in forming an intrachain disulfide bond in the variable region of the immunoglobulin has been replaced with an amino acid residue that does not have a sulfhydryl group and, therefore, does not form a disulfide bond, thereby decreasing the conformational constraints of at least one of the variable regions of the immunoglobulin (Figure 5).

The invention also provides vaccine compositions containing the modified immunoglobulin molecules of the invention. Additionally, the invention provides methods of generating an anti-idiotype response in a subject by administration of the modified immunoglobulin molecules of the invention.

In specific embodiments, the invention provides methods of contraception by administration of a modified immunoglobulin molecule of the invention which, in its unmodified state. is capable of immunospecifically binding an antigen of a protein or cell associated with reproductive function, such as a sperm antigen. Administration of the modified immunoglobulin elicits an anti-idiotype reaction in the subject, leading to the production, by the subject, of antibodies specific for the particular antigen.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

5.1. MODIFIED ANTIBODIES

The modified immunoglobulins, particularly antibodies, of the invention are immunoglobulins that, at least in the unmodified state, can immunospecifically bind an antigen of a cell or protein associated with reproductive function, and have been modified to enhance their ability to elicit an anti-idiotype response. Such immunoglobulins are modified to reduce the conformational constraints on a variable region of the immunoglobulin, *e.g.*, by removing or reducing intrachain or interchain disulfide bonds. Specifically, the invention provides a first immunoglobulin molecule that comprises a variable region and that is identical, except for one or more amino acid substitutions in the variable region, to a second immunoglobulin molecule, the second immunoglobulin molecule being capable of immunospecifically binding an antigen, the amino acid substitutions being the substitution of one or more amino acid residues that do not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule. (See, co-pending United States Patent Application Serial No., entitled "Modified Antibodies With Enhanced Ability To Elicit An Anti-Idiotype Response", filed November 13, 1998 (attorney docket

no. 6750-015), which is incorporated by reference herein in its entirety. The invention also provides nucleic acids containing a nucleotide sequence encoding a modified immunoglobulin of the invention.

Identifying the cysteine residues that form a disulfide bond in a variable region of a particular antibody can be accomplished by any method known in the art. For example, but not by way of limitation, it is well known in the art that the cysteine residues that form intrachain disulfide bonds are highly conserved among antibody classes and across species. Thus, the cysteine residues that participate in disulfide bond formation can be identified by sequence comparison with other antibody molecules in which it is known which residues form a disulfide bond.

Table 1 provides a list of the positions of disulfide bond forming cysteine residues for a number of antibody molecules.

Table 1 (derived from Kabat et al, 1991, sequences of Proteins of Immunological Interest, 5th Ed., U.S. Department of Health and Human Services, Bethesda, Maryland).

Species	Variable domain	Subgroup	Disulfide bond-forming cysteines (positions)
Human	kappa light	I	23,88
Human	kappa light	II	23,88
Human	kappa light	III	23,88
Human	kappa light	IV	23,88
Human	lambda light	I	23,88
Human	lambda light	II	23,88
Human	lambda light	IIII	23,88
Human	lambda light	IV	23,88
Human	lambda light	V	23,88
Human	lambda light	VI	23,88
Mouse	kappa light	I	23,88
Mouse	kappa light	II	23,88
Mouse	kappa light	III	23,88
Mouse	kappa light	IV	23,88
Mouse	kappa light	V	23,88
Mouse	kappa light	VI	23,88
Mouse	kappa light	VII	23,88
Mouse	kappa light	Miscellaneous	23,88
Mouse	lambda light		23,88
Chimpanzee	lambda light		23,88
Rat	kappa light		23,88
Rat	lambda light		23,88
Rabbit	kappa light		23,88
Rabbit	lambda light		23,88

Species	Variable domain	Subgroup	Disulfide bond-forming cysteines (positions)
Dog	kappa light		23,88
Pig	kappa light		23 (88)
Pig	lambda light		23,88
Guinea pig	lambda light		23 (88)
Sheep	lambda light		23,88
Chicken	lambda light		23,88
Turkey	lambda light		23 (88)
Ratfish	lambda light		23 (88)
Shark	kappa light		23,88
Human	heavy	I	22,92
Human	heavy	II	22,92
Human	heavy	III	22,92
Mouse	heavy	I (A)	22,92
Mouse	heavy	I (B)	22,92
Mouse	heavy	II (A)	22,92
Mouse	heavy	II (B)	22,92
Mouse	heavy	II (C)	22,92
Mouse	heavy	III (A)	22,92
Mouse	heavy	III(B)	22,92
Mouse	heavy	III (C)	22,92
Mouse	heavy	III (D)	22,92
Mouse	heavy	V (A)	22,92
Mouse	heavy	V (B)	22,92
Mouse	heavy	Miscellaneous	22,92
Rat	heavy		22,92
Rabbit	heavy		22,92
Guinea pig	heavy		22,92
Cat	heavy		22 (92)
Dog	heavy		22,92
Pig	heavy		22 (92)
Mink	heavy		22 (92)
Sea lion	heavy		22 (92)
Seal	heavy		22 (92)
Chicken	heavy		22,92
Duck	heavy		22 (92)
Goose	heavy		22 (92)
Pigeon	heavy		22 (92)
Turkey	heavy		22 (92)
Caiman	heavy		22, 92
Xenopus frog	heavy		22,92
Elops	heavy		22,92
Goldfish	heavy		22,92
Ratfish	heavy		22 (92)
Shark	heavy		22,92

Position numbers enclosed by () indicate that the protein was not sequenced to that position, but the residue is inferred by comparison to known sequences.

Notably, for all of the antibody molecules listed in Table 1, the cysteine residues that form the intrachain disulfide bonds are the residues at positions 23 and 88 of the light chain variable domain and the residues at positions 22 and 92 of the heavy chain variable domain. The position numbers refer to the residue corresponding to that residue in the consensus sequences as defined in Kabat, (1991, Sequences of Proteins of Immunological Interest, 5th Ed., U.S. Department of Health and Human Services, Bethesda, Maryland) or as indicated in the heavy and light chain variable region sequences depicted in Figures 7A and B, respectively ("corresponding" means as determined by aligning the particular antibody sequence with the consensus sequence or the heavy or light chain variable region sequence depicted in Figure 7A or B).

Accordingly, in one embodiment of the invention, the modified immunoglobulin molecule is an antibody in which the residues at positions 23 and/or 88 of the light chain are substituted with an amino acid residue that does not contain a sulfhydryl group and/or the residues at positions 22 and/or 92 of the heavy chain are substituted with an amino acid residue that does not contain a sulfhydryl group.

In the modified immunoglobulin of the invention, the amino acid residue that substitutes for the disulfide bond forming cysteine residue is any amino acid residue that does not contain a sulfhydryl group, *e.g.*, alanine, arginine, asparagine, aspartate (or aspartic acid), glutamine, glutamate (or glutamic acid), glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine or valine. In a preferred embodiment, the cysteine residue is replaced with a glycine, serine, threonine, tyrosine, asparagine, or glutamine residue, most preferably, with an alanine residue.

Additionally, the disulfide bond forming cysteine residue may be replaced by a nonclassical amino acid or chemical amino acid analog that does not contain a sulfhydryl group (for example, but not by way of limitation, using routine protein synthesis methods). Non-classical amino acids include, but are not limited, to the D-isomers of the common amino acids, α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-aminobutyric acid, γ-Abu, ϵ-Ahx, -amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary). In an alternative embodiment, the disulfide bond forming residue is deleted.

In specific embodiments, the substitution of the disulfide bond forming residue is in the heavy chain variable region or is in the light chain variable region or is in both the heavy chain and light chain variable regions. In other specific embodiments, one of the residues that forms a particular disulfide bond is replaced (or deleted) or, alternatively, both residues that form a particular disulfide bond may be replaced (or deleted).

In other embodiments, the invention provides immunoglobulin molecules that have one or more amino acid substitutions relative to the second immunoglobulin molecule of a disulfide bond forming residue in the variable region with an amino acid residue that does not contain a sulfhydryl group and that additionally have one or more other amino acid substitutions (*i.e.*, that are not a replacement of a disulfide bond forming residue with a residue that does not contain a sulfhydryl group).

In particular, the invention provides a first immunoglobulin molecule comprising a variable region and which is identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding an antigen of a cell or protein associated with reproductive function or that has at least one CDR that contains a portionof an antigen of a cell or protein associated with reproductive function, in which at least one of said one or more amino acid substitutions are the substitution of an amino acid residue that does not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule.

In a preferred embodiment, the amino acid substitutions that are not the substitution of a disulfide bond forming cysteine residue with a residue that does not have a sulfhydryl group, are not stabilizing changes. Stabilizing changes are defined as those amino acid changes that increase the stability of the antibody molecule. Such stabilizing amino acid changes are those changes that substitute an amino acid that is not common at that particular position in the particular antibody molecule (*e.g.*, as defined by the consensus sequences for a number of antibody molecules provided in Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., U.S. Department of Health and Human Services, Bethesda, Maryland) with a residue that is common at that particular position, *e.g.*, is the amino acid at that position in the consensus sequence for that antibody molecule (see PCT Publication WO 96/02574, dated February 1, 1996 by Steipe et al.).

Such other amino acid substitutions can be any amino acid substitution that does not alter the ability of the modified immunoglobulin to elicit the formation of anti-anti-idiotype antibodies, *e.g.*, as determined, for example, as described in Section 5.5, *infra*. For example, such other amino acid substitutions include substitutions of functionally equivalent amino acid residues. For example, one or more amino acid residues can be

substituted by another amino acid of a similar polarity which acts as a functional equivalent. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

The modified immunoglobulin is derived from an antibody that has one or more CDRs containing a portion of an antigen of a cell or protein associated with reproductive function. In specific embodiments, the antigen is a sperm antigen, preferably SP-10. Other antigens include lactate dehydrogenase LDH-C4, SP-17, PH-20, FA-1, FA-2, PH-30, RSA, HAS-63, MSA-63, or zona pellucida proteins ZP1, ZP2, and ZP3 (see, e.g., Freemerman et al., 1993, Molecular Reproduction and Development 34:140-148; Herr et al., 1990, Biol. Reproduction 42:181-193; O'Hern et al., 1995, Biol. Reproduction 52:331-339; Anderson et al., 1986, J. Reprod. Immunol. 10:231-257; Wright et al., 1990, Biology of Reproduction 42:693-701; Lea et al., 1997, Fertility and Sterility 67:355-361; O'Hern et al., Elsevier Science Ltd. 16:1761-1766; Kerr, 1995, Reprod. Fertil. Dev. 7:825-830; Kaul et al., 1996, Reprod. Fertil Dev. 50:127-134; Liu et al., 1990, Molecular Reproduction and Development 25:302-308; Bambra, 1992, Scand. J. Immunol. 11:118-122) or another antigen of a cell or protein associated with reproductive function, for example but not limited to gonadotropin-releasing hormone, any gonadotropin, prostaglandin F2 alpha, oxytocin, and gonadotropin 1 receptors.

The immunoglobulin molecules of the invention can be of any type, class, or subclass of immunoglobulin molecules. In a preferred embodiment, the immunoglobulin molecule is an antibody molecule, more preferably of a type selected from the group consisting of IgG, IgE, IgM, IgD and IgA, most preferably is an IgG molecule. Alternatively, the immunoglobulin molecule is a T cell receptor, a B cell receptor, a cell-surface adhesion molecule such as the co-receptors CD4, CD8, or CD19, or an invariant domain of an MHC molecule.

The modified immunoglobulin can be derived from any naturally occurring antibody, preferably a monoclonal antibody, or can be derived from a synthetic or engineered antibody. Specifically, the modified immunoglobulin molecules are derived from an antibody in which a portion of an antigen of a cell or protein associated with reproductive function is inserted into or replaces all or a portion of one of the CDRs in the variable region, for example as described in co-pending United States Patent application

Serial No., entitled "Immunoglobulin Molecules Having A Synthetic Variable Region And Modified Specificity", by Burch, filed November 13, 1998 (attorney docket no. 6750-016), which is incorporated by reference herein in its entirety.

In particular, the synthetic antibodies are antibodies that in which at least one of the CDRs of the antibody contains an antigen of a cell or protein associated with reproductive function. In one aspect of the invention, the amino acid sequence of the antigen is not found naturally within the CDR. One or more CDRs may also contain a binding site for a cell or protein involved in reproductive function.

The amino acid sequence of the binding site may be identified by any method known in the art. For example, in some instances, the sequence of a member of a binding pair has already been determined to be directly involved in binding the other member of the binding pair. In this case, such a sequence can be used to construct the CDR of a synthetic antibody that specifically recognizes the other member of the binding pair. If the amino acid sequence for the binding site in the one member of the binding pair for the other member of the binding pair is not known, it can be determined by any method known in the art, for example, but not limited to, molecular modeling methods or empirical methods, *e.g.*, by assaying portions (*e.g.*, peptides) of the member for binding to the other member, or by making mutations in the member and determining which mutations prevent binding.

The binding pair can be any two molecules, including proteins, nucleic acids, carbohydrates, or lipids, that interact with each other, although preferably the binding partner from which the binding site is derived is a protein molecule. In preferred embodiments, the modified immunoglobulin contains a binding sequence for a cancer antigen, an infectious disease antigen, a cellular receptor for a pathogen, or a receptor or ligand that participates in a receptor-ligand binding pair.

In specific embodiments, the binding pair is a protein-protein interaction pair which is either homotypic interaction (*i.e.*, is the interaction between two of the same proteins) or a heterotypic interaction (*i.e.*, is the interaction between two different proteins).

The synthetic antibody may be built upon (*i.e.*, the binding site sequences inserted into the CDR of) the sequence of a naturally occurring or previously existing antibody or may be synthesized from known antibody consensus sequences, such as the consensus sequences for the light and heavy chain variable regions in Figures 7A and B, or any other antibody consensus or germline (*i.e.*, unrecombined genomic sequences) sequences (*e.g.*, those antibody consensus and germline sequences described in Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th edition, NIH Publication No. 91-3242, pp 2147-2172).

Each antibody molecule has six CDR sequences, three on the light chain and three on the heavy chain, and five of these CDRs are germline CDRs (*i.e.*, are directly derived from the germline genomic sequence of the animal, without any recombination) and one of the CDRs is a non-germline CDR (*i.e.*, differs in sequence from the germline genomic sequence of the animal and is generated by recombination of the germline sequences). Whether a CDR is a germline or non-germline sequence can be determined by sequencing the CDR and then comparing the sequence with known germline sequences, *e.g.*, as listed in Kabat et al. (1991, Sequences of Proteins of Immunological Interest, 5th edition, NIH Publication No. 91-3242, pp 2147-2172). Significant variation from the known germline sequences indicates that the CDR is a non-germline CDR. Accordingly, the CDR that contains the amino acid sequence of the binding site or antigen is a germline CDR or, alternatively, is a non-germline CDR.

The binding site or antigen sequence can be inserted into any of the CDRs of the antibody, and it is within the skill in the art to insert the binding site into different CDRs of the antibody and then screen the resulting modified antibodies for the ability to bind to the particular member of the binding pair, *e.g.* as discussed in Section, *infra*, or to elicit an immune response against the antigenic site, *e.g.*, as described in Section, *infra*. Thus, one can determine which CDR optimally contains the binding site or antigen. In specific embodiments, a CDR of either the heavy or light chain variable region is modified to contain the amino acid sequence of the binding site or antigen. In another specific embodiment, the modified antibody contains a variable domain in which the first, second or third CDR of the heavy variable region or the first, second or third CDR of the light chain variable region contains the amino acid sequence of the binding site or antigen. In another embodiment of the invention, more than one CDR contains the amino acid sequence of the binding site or antigen or more than one CDR each contains a different binding site for the same molecule or contains a different binding site for a different molecule. In particular, embodiments, two, three, four, five or six CDRs have been engineered to contain a binding site for the first member of the binding pair. In a preferred embodiment, one of the CDRs contains a portion of one sperm antigen and another CDR contains a portion of a second sperm antigen, more particularly, where one sperm antigen in SP-10 and the other sperm antigen is MSA-63 or LHD-C_4.

In specific embodiments of the invention, the binding site or antigen amino acid sequence is either inserted into the CDR without replacing any of the amino acid sequence of the CDR itself or, alternatively, the binding site or antigen amino acid sequence replaces all or a portion of the amino acid sequence of the CDR. In specific embodiments, the

binding site amino acid sequence replaces 1, 2, 5, 8, 10, 15, or 20 amino acids of the CDR sequence.

The amino acid sequence of the binding site or antigen present in the CDR can be the minimal binding site necessary for the binding of the member of the binding pair or for eliciting an immune response against the antigen(which can be determined empirically by any method known in the art); alternatively, the sequence can be greater than the minimal binding site or antigen sequence necessary for the binding of the member of the binding pair or eliciting of an immune response against the antigen. In particular embodiments, the binding site or antigen amino acid sequence is at least 4 amino acids in length, or is at least 6, 8, 10, 15, or 20 amino acids in length. In other embodiments the binding site amino acid sequence is no more than 10, 15, 20, or 25 amino acids in length, or is 5-10, 5-15, 5-20, 10-15, 10-20 or 10-25 amino acids in length.

In addition, the total length of the CDR (*i.e.*, the combined length of the binding site sequence and the rest of the CDR sequence) should be of an appropriate number of amino acids to allow binding of the antibody to the antigen. CDRs have been observed to have a range of numbers of amino acid residues, and the observed size ranges for the CDRs (as denoted by the abbreviations indicated in figure 2) are provided in Table 1.

Table 1

CDR	Number of residues
L1	10-17
L2	7
L3	7-11
H1	5-7
H2	9-12
H3	2-25

(compiled from data in Kabat and Wu, 1971, *Ann. NY Acad. Sci.* 190:382-93)

While many CDR H3 regions are of 5-9 residue in length, certain CDR H3 regions have been observed that are much longer. In particular, a number of antiviral antibodies have heavy chain CDR H3 regions of 17-24 residues in length.

Accordingly, in specific embodiments of the invention, the CDR containing the binding site or antigen portion is within the size range provided for that particular CDR in Table 1, *i.e.*, if it is the first CDR of the light chain, L1, the CDR is 10 to 17 amino acid residues; if it is the second CDR of the light chain, L2, the CDR is 7 amino acid residues; if it is the third CDR of the light chain, L3, the CDR is 7 to 11 amino acid residues; if it is the

first CDR of the heavy chain, H1, the CDR is 5 to 7 amino acid residues; if it is the second CDR of the heavy chain, H2, the CDR is 9 to 12 amino acid residues; and if it is the third CDR of the heavy chain, H3, the CDR is 2 to 25 amino acid residues. In other specific embodiments, the CDR containing the binding site is 5-10, 5-15, 5-20, 11-15, 11-20, 11-25, or 16-25 amino acids in length. In other embodiments, the CDR containing the binding site is at least 5, 10, 15, or 20 amino acids or is no more than 10, 15, 20, 25, or 30 amino acids in length.

After constructing antibodies containing modified CDRs, the modified antibodies can be further altered and screened to select an antibody having higher affinity or specificity. Antibodies having higher affinity or specificity for the target antigen may be generated and selected by any method known in the art. For example, but not by way of limitation, the nucleic acid encoding the synthetic modified antibody can be mutagenized, either randomly, *i.e.*, by chemical or site-directed mutagenesis, or by making particular mutations at specific positions in the nucleic acid encoding the modified antibody, and then screening the antibodies exposed from the mutated nucleic acid molecules for binding affinity for the target antigen. Screening can be accomplished by testing the expressed antibody molecules individually or by screening a library of the mutated sequences, *e.g.*, by phage display techniques (see, *e.g.*, U. S. Patent Nos. 5,223,409; 5,403,484; and 5,571,698, all by Ladner et al; PCT Publication WO 92/01047 by McCafferty et al. or any other phage display technique known in the art).

In specific embodiments, the invention provides a functionally active fragment, derivative or analog of the modified immunoglobulin molecules of the invention. Functionally active means that the fragment, derivative or analog is able to elicit anti-anti-idiotype antibodies (*i.e.*, tertiary antibodies or Ab3 antibodies) that recognize the same antigen that the antibody from which the fragment, derivative or analog is derived recognized (*e.g.*, as determined by the methods described in Section 5.4, *infra*). Specifically, in a preferred embodiment, the antigenicity of the idiotype of the immunoglobulin molecule may be enhanced by deletion of framework and CDR sequences that are N-terminal to the particular CDR sequence that specifically recognizes the antigen. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR sequences can be used in binding assays with the antigen by any binding assay method known in the art. Accordingly, in a preferred embodiment, the invention includes modified immunoglobulin molecules that have one disulfide bond forming cysteine residue in a variable region domain replaced with an amino acid residue that does not contain a sulfhydryl group and in which a portion of that variable domain has been deleted N-terminal to the CDR sequence that recognizes the antigen.

Other embodiments of the invention include fragments of the modified antibodies of the invention such as, but not limited to, $F(ab')_2$ fragments, which contain the variable region, the light chain constant region and the CH1 domain of the heavy chain can be produced by pepsin digestion of the antibody molecule, and Fab fragments, which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. The invention also provides heavy chain and light chain dimers of the modified antibodies of the invention, or any minimal fragment thereof such as Fvs or single chain antibodies (SCAs) (*e.g.*, as described in U.S. Patent 4,946,778; Bird, 1988, Science 242:423-42; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54), or any other molecule with the same specificity as the modified antibody of the invention.

Techniques have been developed for the production of "chimeric antibodies" (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a constant domain from a human immunoglobulin, *e.g.*, humanized antibodies.

In a preferred embodiment, the modified immunoglobulin of the invention is a humanized antibody, more preferably an antibody having a variable domain in which the framework regions are from a human antibody and the CDRs are from an antibody of a non-human animal, preferably a mouse (see, International Patent Application No. PCT/GB8500392 by Neuberger et al. and Celltech Limited).

CDR grafting is another method of humanizing antibodies. It involves reshaping murine antibodies in order to transfer full antigen specificity and binding affinity to a human framework (Winter et al. U.S. Patent No. 5,225,539). CDR-grafted antibodies have been successfully constructed against various antigens, for example, antibodies against IL-2 receptor as described in Queen et al., 1989 (Proc. Natl. Acad. Sci. USA 86:10029); antibodies against cell surface receptors-CAMPATH as described in Riechmann et al. (1988, Nature, 332:323); antibodies against hepatitis B in Cole et al. (1991, Proc. Natl. Acad. Sci. USA 88:2869); as well as against viral antigens-respiratory syncitial virus in Tempest et al. (1991, Bio-Technology 9:267). CDR-grafted antibodies are generated in which the CDRs of the murine monoclonal antibody are grafted into a human antibody. Following grafting, most antibodies benefit from additional amino acid changes in the framework region to maintain affinity, presumably because framework residues are necessary to maintain CDR conformation, and some framework residues have been

demonstrated to be part of the antigen binding site. However, in order to preserve the framework region so as not to introduce any antigenic site, the sequence is compared with established germline sequences followed by computer modeling.

In other embodiments, the invention provides fusion proteins of the modified immunoglobulins of the invention (or functionally active fragments thereof), for example in which the modified immunoglobulin is fused via a covalent bond (*e.g.*, a peptide bond), at either the N-terminus or the C-terminus to an amino acid sequence of another protein (or portion thereof, preferably an at least 10, 20 or 50 amino acid portion of the protein) that is not the modified immunoglobulin. Preferably the modified immunoglobulin, or fragment thereof, is covalently linked to the other protein at the N-terminus of the constant domain. In preferred embodiments, the invention provides fusion proteins in which the modified immunoglobulin is covalently linked to IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, γ-interferon, MHC derived peptide, G-CSF, a porin, TNF, NK cell antigens, or cellular endocytosis receptor.

The modified immunoglobulins of the invention include analogs and derivatives that are either modified, *i.e*, by the covalent attachment of any type of molecule as long as such covalent attachment does not prevent the modified immunoglobulin from generating an anti-idiotypic response (*e.g.*, as determined by any of the methods described in Section 5.5, *infra*). For example, but not by way of limitation, the derivatives and analogs of the modified immunoglobulins include those that have been further modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the analog or derivative may contain one or more non-classical amino acids, *e.g.*, as listed above in this Section.

Methods of producing the modified immunoglobulins, and fragments, analogs, and derivatives thereof, are described in Section 5.4, *infra*.

5.2. CONTRACEPTIVE METHODS

The present invention provides methods of contraception by eliciting production of anti-idiotype antibodies and anti-anti-idiotype antibodies in a subject by the administration of a therapeutic (termed herein "Therapeutic"). Such Therapeutics include the modified immunoglobulins of the invention, and functionally active fragments, analogs, and derivatives thereof (*e.g.*, as described in Section 5.1, *supra*), and nucleic acids encoding the

modified antibodies of the invention, and functionally active fragments and derivatives thereof (*e.g.*, as described in Section 5.1, *supra*).

Generally, administration of products of a species origin or species reactivity that is the same species as that of the subject is preferred. Thus, in a preferred embodiment, the methods of the invention use a modified antibody that is derived from a human antibody; in other embodiments, the methods of the invention use a modified antibody that is derived from a chimeric or humanized antibody.

Specifically, vaccine compositions (*e.g.*, as described in Section 5.3, *infra*) containing the modified antibodies of the invention are administered to the subject to elicit the production of an antibody (*i.e.*, the anti-idiotype antibody or Ab2) that specifically recognizes the idiotype of the modified antibody, the Ab2, in turn, elicits the production anti-anti-idiotype antibodies (Ab3) that specifically recognize the idiotype of Ab2, such that these Ab3 antibodies have the same or similar binding specificity as the modified antibody.

The invention provides methods of administering the modified antibodies of the invention to elicit an anti-idiotype response, *i.e.*, to generate Ab2 and Ab3 type antibodies. Alternatively, the invention provides methods of administering the modified antibodies of the invention to one subject to generate Ab2 antibodies, isolating the Ab2 antibodies, and then administering the Ab2 antibodies to a second subject to generate Ab3 type antibodies in that second subject.

Accordingly, the invention provides a method of generating an anti-idiotype response in a subject comprising administering an amount of first immunoglobulin molecule (or functionally active fragment, analog, or derivative thereof) sufficient to induce an anti-idiotype response, said first immunoglobulin comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding an antigen, said one or more amino acid substitutions being the substitution of an amino acid residue that does not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule. In another embodiment, the method further provides isolating the anti-idiotype antibody that recognizes the idiotype of said second immunoglobulin molecule, and administering to a second subject the anti-idiotype antibody.

Modified immunoglobulins capable of inhibiting the gamete interaction *i.e.*, of eggs and sperm are preferably employed. The key to this method of contraception is to either immunologically regulate molecules involved in reproduction or to inhibit fertilization. Such contraceptive vaccines target reproductive hormone or receptor-specific antigens or gamete-specific antigens. The goal is to elicit an immune response which targets

reproductive hormones or receptors or native gamete molecules. In prefered embodiments, the vaccine targets sperm by eliciting production of antibodies that recognize sperm antigens.

Fertility can be suppressed by immunization against a reproductive hormone or receptor such as gonadotropin-releasing hormone, gonadotropins, prostaglandin F2 alpha, oxytocin and gonadotropin receptors.

Fertility can also be suppressed by immunization against gamete or embryonic antigens. Fertilization is mediated through specific molecules of the sperm and egg. In mammals, the sperm and egg interact at an egg-specific extracellular matrix, the zona pellucida (ZP), and the sperm plasma membrane (Gupta et al., 1997, Hum. Reprod. Update, 3(4):311-324). The zona pellucida comprises three glycoproteins ZP1, ZP2 and ZP3 (Kaul et al., 1997, Mol. Reprod. Dev. 47(2):140-147) which are target antigens for designing immunocontraceptives. Some of the sperm plasma membrane proteins which are useful as antigens for immunocontraception are PH-20 (Primakoff et al., 1997, Biol. Reprod., 56(5):1142-1146) and PH-30 (Kerr, Reprod. Fertil. Dev., 1995, 7(4):825-830). Other sperm proteins are SP-10 (Kurth et al., 1997, Biol. Reprod., 57(5):981-989) and SP-17 (Adoyo et al., 1997, Mol. Reprod. Dev., 47(1):66-71). Other gamete proteins include lactate dehydrogenase-C4 (LDH-C4) (Bradley et al., Reprod. Fertil. Dev., 9(1):111-116) and fertilization antigen-1 (FA-1) (Zhu and Naz, Proc. Natl. Acad. Sci. USA., 94(9):4704-4709).

In particular, the contraceptive methods of the invention involve administration of modified immunoglobulin molecules (or functionally active fragments, derivatives or an analog thereof, or nucleic acids encoding the same) derived from an immunoglobulin molecule that specifically recognizes a molecule or cell involved in reproductive function. In a specific embodiment, the contraceptive methods of the invention involve the administration of a modified immunoglobulin molecule that is derived from an antibody that is capable of immunospecifically binding to gonadotropin-releasing hormone, any gonadotropin, prostaglandin F2 alpha, oxytocin, gonadotropin receptors, gamete or embryonic antigens, sperm antigens, preferably SP-10. Other antigens include, but are not limited to, lactate dehydrogenase LDH-C4, SP-17, PH-20, FA-1, FA-2, PH-30, RSA, HAS-63, MSA-63, or zona pellucida proteins ZP1, ZP2, and ZP3 (see, e.g., Freemerman et al., 1993, Molecular Reproduction and Development 34:140-148; Herr et al., 1990, Biol. Reproduction 42:181-193; O'Hern et al., 1995, Biol. Reproduction 52:331-339; Anderson et al., 1986, J. Reprod. Immunol. 10:231-257; Wright et al., 1990, Biology of Reproduction 42:693-701; Lea et al., 1997, Fertility and Sterility 67:355-361; O'Hern et al., Elsevier Science Ltd. 16:1761-1766; Kerr, 1995, Reprod. Fertil. Dev. 7:825-830; Kaul et al., 1996,

Reprod. Fertil Dev. 50:127-134; Liu et al., 1990, Molecular Reproduction and Development 25:302-308; Bambra, 1992, Scand. J. Immunol. 11:118-122).

The invention also includes contraceptive methods whereby a modified immunoglobulin of the invention is administered in conjunction with use of another contraceptive method, such as, but not limited to, barrier methods such as the use of condoms or diaphragms or cervical caps, or intravaginal use of contraceptive compounds such as, but not limited to, non-oxynol-9, intrauterine devices, birth control pills or implants, etc.

The invention also includes administrations of anti-anti-idiotype antibodies against a modified immunoglobulin of the invention to acutely neutralize the contraceptive activity of the modified immunoglobulin.

The methods and vaccine compositions of the present invention may be used to elicit a humoral and/or a cell-mediated response against a modified immunoglobulin in a subject. In one specific embodiment, the methods and compositions of the invention elicit a humoral response in a subject. In another specific embodiment, the methods and compositions of the invention elicit a cell-mediated response in a subject. In a preferred embodiment, the methods and compositions of the invention elicit both a humoral and a cell-mediated response.

The subjects to which the present invention is applicable may be any mammalian or vertebrate species, which include, but are not limited to, cows, horses, sheep, pigs, fowl (*e.g.*, chickens), goats, cats, dogs, hamsters, mice, rats, monkeys, rabbits, chimpanzees, and humans. In a preferred embodiment, the subject is a human.

5.2.1. GENE THERAPY

Gene therapy may be used by administering a nucleic acid containing a nucleotide sequence encoding the modified immunoglobulin of the invention as a contraceptive. In this embodiment of the invention, the therapeutic nucleic acid encodes a sequence that produces intracellularly (without a leader sequence) or intercellularly (with a leader sequence), a modified immunoglobulin.

For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, *Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; Kriegler,

1990, *Gene Transfer and Expression. A Laboratory Manual*. Stockton Press, NY; and in Chapters 12 and 13. Dracopoli et al. (eds), 1994, *Current Protocols in Human Genetics*, John Wiley & Sons, NY).

In one aspect, the therapeutic nucleic acid comprises an expression vector that expresses the modified immunoglobulin molecule.

Delivery of the nucleic acid into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vector or a delivery complex, or indirect, in which case, cells are first transformed with the nucleic acid *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid is directly administered *in vivo*, where it is expressed to produce the antibodies. This can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by infection using a defective or attenuated retroviral or other viral vector (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in biopolymers (*e.g.*, poly-ß-1->4-N-acetylglucosamine polysaccharide; see U.S. Patent No. 5,635,493), encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see *e.g.*, Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432), etc. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, PCT Publications WO 92/06180 dated April 16, 1992 (Wu et al.); WO 92/22635 dated December 23, 1992 (Wilson et al.); WO92/20316 dated November 26, 1992 (Findeis et al.); WO93/14188 dated July 22, 1993 (Young). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra et al., 1989, *Nature* 342:435-438).

Alternatively, single chain antibodies, such as neutralizing antibodies, which bind to intracellular epitopes can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population by utilizing, for example, techniques such as

those described in Marasco et al. (Marasco et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:7889-7893). Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 1993, *Current Opinion in Genetics and Development* 3:499-503 present a review of adenovirus-based gene therapy. Bout et al., 1994, *Human Gene Therapy* 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., 1991, *Science* 252:431-434; Rosenfeld et al., 1992, *Cell* 68:143-155; and Mastrangeli et al., 1993, *J. Clin. Invest.* 91:225-234. Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, *Proc. Soc. Exp. Biol. Med.* 204:289-300).

The form and amount of therapeutic nucleic acid envisioned for use depends on the type of disease and the severity of its desired effect, patient state, etc., and can be determined by one skilled in the art.

5.3. VACCINE FORMULATIONS AND ADMINISTRATION

The invention also provides vaccine formulations containing Therapeutics of the invention, which vaccine formulations are suitable for administration to elicit a protective immune (humoral and/or cell mediated) response against certain antigens, *e.g.*, for the contraceptive uses described herein. .

Suitable preparations of such vaccines include injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection, may also be prepared. The preparation may also be emulsified, or the polypeptides encapsulated in liposomes. The active immunogenic ingredients are often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, buffered saline, dextrose, glycerol, ethanol, sterile isotonic aqueous buffer or the like and combinations thereof. In addition, if desired, the vaccine preparation may also include minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine.

Examples of adjuvants which may be effective, include, but are not limited to: aluminim hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine.

The effectiveness of an adjuvant may be determined by measuring the induction of anti-idiotype antibodies directed against the injected immunoglobulin formulated with the particular adjuvant.

The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is administered by injection, an ampoule of sterile diluent can be provided so that the ingredients may be mixed prior to administration.

In a specific embodiment, the lyophilized modified immunoglobulin of the invention is provided in a first container; a second container comprises diluent consisting of an aqueous solution of 50% glycerin, 0.25% phenol, and an antiseptic (*e.g.*, 0.005% brilliant green).

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the vaccine formulations of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Composition comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

The subject to which the vaccine is administered is preferably a mammal, most preferably a human, but can also be a non-human animal including but not limited to cows, horses, sheep, pigs, fowl (*e.g.*, chickens), goats, cats, dogs, hamsters, mice and rats.

Many methods may be used to introduce the vaccine formulations of the invention; these include but are not limited to oral, intracerebral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal routes, and via scarification

(scratching through the top layers of skin, *e.g.*, using a bifurcated needle) or any other standard routes of immunization. In a specific embodiment, scarification is employed.

The precise dose of the modified immunoglobulin molecule to be employed in the formulation will also depend on the route of administration, and the nature of the patient, and should be decided according to the judgment of the practitioner and each patient's circumstances according to standard clinical techniques. An effective immunizing amount is that amount sufficient to produce an immune response to the modified immunoglobulin molecule in the host (*i.e.*, an anti-idiotype reaction) to which the vaccine preparation is administered. Effective doses may also be extrapolated from dose-response curves derived from animal model test systems.

5.4. METHOD OF PRODUCING THE MODIFIED IMMUNOGLOBULINS

The modified immunoglobulins of the invention can be produced by any method known in the art for the synthesis of immunoglobulins, in particular, by chemical synthesis or by recombinant expression, and is preferably produced by recombinant expression techniques.

Recombinant expression of the modified immunoglobulin of the invention, or fragment, derivative or analog thereof, requires construction of a nucleic acid that encodes the modified immunoglobulin. If the nucleotide sequence of the modified immunoglobulin is known, a nucleic acid encoding the modified immunoglobulin may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier et al., 1994, *BioTechniques* 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the modified immunoglobulin, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR, *e.g.*, as exemplified in Section 6, *infra*.

Alternatively, the nucleic acid encoding the modified immunoglobulin may be generated from a nucleic acid encoding the immunoglobulin from which the modified immunoglobulin was derived. If a clone containing the nucleic acid encoding the particular immunoglobulin is not available, but the sequence of the immunoglobulin molecule is known, a nucleic acid encoding the immunoglobulin may be obtained from a suitable source (*e.g.*, an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the immunoglobulin) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by hybridization using an oligonucleotide probe specific for the particular gene sequence.

If an immunoglobulin molecule that specifically recognizes a particular antigen is not available (or a source for a cDNA library for cloning a nucleic acid encoding such an

immunoglobulin is not available), immunoglobulins specific for a particular antigen may be generated by any method known in the art, for example, by immunizing an animal, such as a rabbit, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies, *e.g.*, as described by Kohler and Milstein (1975, *Nature* 256:495-497) or, as described by Kozbon et al. (1983, *Immunology Today* 4:72) or Cole et al. (1985 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Alternatively, a clone encoding at least the Fab portion of the immunoglobulin can be obtained by screening Fab expression libraries (*e.g.*, as described in Huse et al., 1989, *Science* 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (see, *e.g.*, Clackson et al., 1991, Nature 352:624; Hane et al., 1997 Proc. Natl. Acad. Sci. USA 94:4937).

Once a nucleic acid encoding at least the variable domain of the immunoglobulin molecule is obtained, it may be introduced into any available cloning vector, and may be introduced into a vector containing the nucleotide sequence encoding the constant region of the immunoglobulin molecule (see, *e.g.*, PCT Publication WO 86/05807; PCT Publication WO 89/01036; U.S. Patent No. 5,122,464; and Bebbington, 1991, Methods in Enzymology 2:136-145). Vectors containing the complete light or heavy chain for co-expression with the nucleic acid to allow the expression of a complete antibody molecule are also available, see *Id.* Then, the nucleic acid encoding the immunoglobulin can be modified to introduce the nucleotide substitutions or deletion necessary to substitute (or delete) the one or more variable region cysteine residues participating in an intrachain disulfide bond with an amino acid residue that does not contain a sulfhydyl group, along with any other desired amino acid substitutions, deletions or insertions. Such modifications can be carried out by any method known in the art for the introduction of specific mutations or deletions in a nucleotide sequence, for example, but not limited to, chemical muagenesis, *in vitro* site directed mutagenesis (Hutchinson et al., 1978, *J. Biol. Chem.* 253:6551), PCR based methods, etc.

In addition, techniques developed for the production of chimeric antibodies (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can also be used. As described *supra*, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a constnat region derived from a human immunoglobulin, *e.g.*, humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,694,778; Bird, 1988, *Science* 242:423-42; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., 1988, *Science* 242:1038-1041).

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments.

Once a nucleic acid encoding the modified immunoglobulin molecule of the invention has been obtained, the vector for the production of the immunoglobulin molecule may be produced by recombinant DNA technology using techniques well known in the art. The modified immunoglobulin molecule can then be recombinantly expressed and isolated by any method known in the art, for example, using the method described in Section 6, *supra*, (see also Bebbington, 1991, Methods in Enzymology 2:136-145). Briefly, COS cells, or any other appropriate cultured cells, can be transiently or non-transiently transfected with the expression vector encoding the modified immunoglobulin, cultured for an appropriate period of time to permit immunoglobulin expression, and then the supernatan can be harvested from the COS cells, which supernatant contains the secreted, expressed modified immunoglobulin.

Methods which are well known to those skilled in the art can be used to construct expression vectors containing the immunoglobulin molecule coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. See, for example, the techniques described in Sambrook et al. (1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and Ausubel et al. (eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY).

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce the immunoglobulin of the invention.

The host cells used to express the recombinant antibody of the invention may be either bacterial cells such as *Escherichia coli*, or, preferably, eukaryotic cells, especially for

the expression of whole recombinant immunoglobulin molecules. In particular, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for immunoglobulins (Foecking et al., 198, *Gene* 45:101; Cockett et al., 1990, *Bio/Technology* 8:2).

A variety of host-expression vector systems may be utilized to express the modified immunoglobulin molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express the immunoglobulin molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (*e.g.*, *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing immunoglobulin coding sequences; yeast (*e.g.*, *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing immunoglobulin coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the immunoglobulin coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing immunoglobulin coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the immunoglobulin molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an immunoglobulin molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, *EMBO J.* 2:1791), in which the immunoglobulin coding sequence may be ligated individually into the vector in frame with the *lac Z* coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-

agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system. *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The immunoglobulin coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the immunoglobulin coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the immunoglobulin molecule in infected hosts (*e.g.*, see Logan & Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:355-359). Specific initiation signals may also be required for efficient translation of inserted immunoglobulin coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, *Methods in Enzymol.* 153:51-544).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the immunoglobulin molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the immunoglobulin molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the immunoglobulin molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., 1977, *Cell* 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 192, *Proc. Natl. Acad. Sci. USA* 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell* 22:817) genes can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., 1980, *Natl. Acad. Sci. USA* 77:357; O'Hare et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072); neo, which confers resistance to the aminoglycoside G-418Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11(5):155-215). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY; and in Chapters 12 and 13. Dracopoli et al. (eds), 1994, Current Protocols in Human Genetics, John Wiley & Sons, NY.; Colberre-Garapin et al., 1981, *J. Mol. Biol.* 150:1.

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:8972-897). In

this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+}-nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

The expression levels of the immunoglobulin molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, *the Use of Vectors Based on Gene Amplification for the Expression of Cloned Genes in Mammalian Cells in DNA Cloning*, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing immunoglobulin is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the immunoglobulin gene, production of the immunoglobulin will also increase (Crouse et al., 1983, *Mol. Cell. Biol.* 3:257).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, *Nature* 322:52; Kohler, 1980, *Proc. Natl. Acad. Sci. USA* 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once the modified immunoglobulin molecule of the invention has been recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

5.5. DEMONSTRATION OF THERAPEUTIC UTILITY

The modified antibodies of the invention can be screened or assayed in a variety of ways for efficacy in treating or preventing a particular disease .

First, the immunopotency of a vaccine formulation containing the modified antibody of the invention can be determined by monitoring the anti-idiotypic response of test animals following immunization with the vaccine. Generation of a humoral response may be taken

as an indication of a generalized immune response, other components of which, particularly cell-mediated immunity, may also be important. Test animals may include mice, rabbits, chimpanzees and eventually human subjects. A vaccine made in this invention can be made to infect chimpanzees experimentally. However, since chimpanzees are a protected species, the antibody response to a vaccine of the invention can first be studied in a number of smaller, less expensive animals, with the goal of finding one or two best candidate immunoglobulin molecules or best combinations of immunoglobulin molecules to use in chimpanzee efficacy studies.

The immune response of the test subjects can be analyzed by various approaches such as the reactivity of the resultant immune serum to antibodies, as assayed by known techniques, *e.g.*, enzyme linked immunosorbent assay (ELISA), immunoblots, radioimmunoprecipitations, etc.; or protection from infection and/or attenuation of disease symptoms in immunized hosts.

As one example of suitable animal testing, the vaccine composition of the invention may be tested in rabbits for the ability to induce an anti-idiotypic response to the modified immunoglobulin molecule. For example, male specific-pathogen-free (SPF) young adult New Zealand White rabbits may be used. The test group of rabbits each receives an effective amount of the vaccine. A control group of rabbits receives an injection in 1 mM Tris-HCl pH 9.0 of the vaccine containing a naturally occurring antibody. Blood samples may be drawn from the rabbits every one or two weeks, and serum analyzed for anti-idiotypic antibodies to the modified immunoglobulin molecule and anti-anti-idiotypic antibodies specific for the antigen against which the modified antibody was directed using, *e.g.*, a radioimmunoassay (Abbott Laboratories). The presence of anti-idiotypic antibodies may be assayed using an ELISA. Because rabbits may give a variable response due to their outbred nature, it may also be useful to test the vaccines in mice.

In addition, a modified antibody of the invention may be tested by first administering the modified antibody to a test subject, either animal or human, and then isolating the anti-anti-idiotypic antibodies (*i.e.*, the Ab3 antibodies) generated as part of the anti-idiotype response to the injected modified antibody. The isolated Ab3 may then be tested for the ability to bind the particular antigen (*e.g.*, a tumor antigen, antigen of an infectious disease agent by any immunoassays known in the art, for example, but not limited to, radioimmunoassays, ELISA, "sandwich" immunoassay, gel diffusion precipitin reactions, immunodiffusion assays, western blots, precipitation reactions, agglytination assays, complement fixation assays, immunofluorescence assays, protein A assays, immunoelectrophoresis assays, etc.

Additionally, the modified antibodies of the invention may also be tested directly *in vivo*. The strength of the immune response *in vivo* to the modified immunogluobulin may be determined by any method known in the art, for example, but not limited to, delayed hypersensitivity skin tests and assays of the activity of cytolytic T-lymphocytes *in vitro*.

Delayed hypersensitivity skin tests are of great value in the testing of the overall immunocompetence and cellular immunity to an antigen. Proper technique of skin testing requires that the antigens be stored sterile at 4°C, protected from light and reconstituted shortly before use. A 25- or 27-gauge need ensures intradermal, rather than subcutaneous, administration of antigen. Twenty-four and 48 hours after intradermal administration of the antigen, the largest dimensions of both erythema and induration are measured with a ruler. Hypoactivity to any given antigen or group of antigens is confirmed by testing with higher concentrations of antigen or, in ambiguous circumstances, by a repeat test with an intermediate test.

To test the activity of cytolytic T-lymphocytes, T-lymphocytes isolated from the immunized subject, *e.g.*, by the Ficoll-Hypaque centrifugation gradient technique, are restimulated with cells bearing the antigen against which the modified antibody was directed in 3 ml RPMI medium containing 10% fetal calf serum. In some experiments, 33% secondary mixed lymphocyte culture supernatant or IL-2 is included in the culture medium as a source of T cell growth factors. In order to measure the primary response of cytolytic T-lymphocytes after immunization, the isolated T cells are cultured with or without the cells bearing the antigen. After six days, the cultures are tested for cytotoxity in a 4 hour ^{51}Cr-release assay. The spontaneous ^{51}Cr-release of the targets should reach a level less than 20% if immunization was effective (Heike et al., *J. Immunotherapy* 15:15-174).

The efficacy of the modified antibody as a contraceptive can also be tested by any method known for tested contraceptive methods. For example, a vaccine composition containing a modified antibody of the invention specific for an antigen of a protein or cell involved in reproductive function. First, the level of the particular antigen in the subject can be measured by any method known in the art where a reduction in the level of the antigen compared to the level prior to administration of the modified antibody (accounting for normal, cyclical changes of the level of the particular antigen) indicates that the modified antibody may be effective. The modified antibody must then be administered to a population of child bearing age (and having partners of childbearing age) and the percentage of females that conceive over a suitable period of time is determined. If the number of females that conceive is significantly lower than those in a control population, *e.g.*, those administered a placebo or not using a contraceptive method, indicates that the modified antibody is effective as a contraceptive.

Additionally, the efficacy of the contraceptive vaccine may be assayed by administering the vaccine to a subject or animal model, allowing an appropriate amount of time for the production of anti-idiotype antibodies, and then testing serum taken from the subject or animal for the ability to bind the particular antigen (indicating that an anti-idiotype reaction has occurred) and/or testing whether the serum can block fertilization in vitro, which can be tested by any method known in the art, for example as described in Brannen-Brock et al., 1985, Arch. Androl. 15:15-19. .

6. EXAMPLE: ANTI-IDIOTYPIC VACCINE INDUCER FOR COLON CANCER

This example describes the construction of a modified antibody derived from the monoclonal antibody MAb31.1 (hybridoma secreting Mab31.1 is available from the American Type Tissue Collection as accession No. HB12314). Mab31.1 recognizes an antigen expressed by human colon carcinomas. The modified antibody of the invention, based on Mab31.1, was engineered to have variable region cysteine residues of both the heavy and light chain variable regions substituted with alanine. Therefore, the resulting modified antibody, was missing intrachain disulfide bonds in either the heavy and light chain variable regions.

6.1. CONSTRUCTION OF A MODIFIED ANTIBODY

The strategy for construction of the modified antibody was to construct two engineered genes that encoded the heavy and light chain variable regions wherein specific cysteine residues, known to be important in intra-chain disulfide bonding , were altered to alanine. Alanine residues were substituted for the cysteine residues at positions 22 and 92 of the heavy chain variable region of the antibody derived from Mab31.1 or at positions 23 and 88 of the Mab31.1 light chain variable region of the antibody derived from Mab31.1. In order to construct these engineered genes, groups of olionucleotides were assembled (as discussed below) and inserted into an appropriate vector providing constant regions.

In order to construct variable region genes encoding CDRs lacking intrachain disulfide bonds, the following strategy was performed.

First, single strand oligonucleotides were annealed to create cohesive double stranded DNA fragments (as diagramed in Figure 8, Step 1). Specifically, oligonucleotides of about 80 bases in length corresponding to the sequences of interest with 20 base overlapping regions were synthesized using automated techniques of GenoSys Biotech Inc. The specific sequences of each of these oligonucleotides. The specific sequences of these

oligonucleotides are presented in Figures 16A and 16B. Figure 16A list the group of ten oligos used in engineering a heavy chain variable region gene called 2CAVHCOL1. 2CAVHCOL1 lacked 2 cysteine residues as compared to the consensus heavy chain variable gene. Figure 16B lists the group of 12 oligos used in the engineering of the light chain variable region gene called 2CAVLCOL1. 2CAVLCOL1 lacked two cysteine residues as compared to the consensus light chain variable region gene. In order to combine the oligos into the desired gene, groups of 10 or 12 oligos were combined as described below and as presented in Figure 8, where the identities of oligos 1 to 10 indicated in Figure 8 are provided in Table 5. Prior to combining, each oligonucleotide was 5' phosphorylated as follows: 25µl of each oligo was incubated for 1 hour in the presence of T4 polynucleotide kinase and 50mM ATP at 37°C. The reactions were stopped by heating for 5 minutes at 70°C followed by ethanol precipitation. Once phosphorylated, complementary oligonucleotides (oligo 1 + oligo 10, oligo 2 + oligo 9, oligo 3 + oligo 8, oligo 4 + oligo 7, oligo 5 + oligo 6), as shown in Figure 8, were then mixed in sterile microcentrifuge tubes and annealed by heating the tube in a water bath at 65°C for 5 minutes followed by cooling at room temperature for 30 minutes. Annealing resulted in short double strand DNA fragments with cohesive ends.

Next, the cohesive double stand DNA fragments were ligated into longer strands (Figure 8, Steps 2-4), until the engineered variable region gene was assembled. Specifically, cohesive double strand DNA fragments were ligated in the presence of T4 DNA ligase and 10mM ATP for 2 hours in a water bath maintained at 16°C. Annealed oligo 1/10 was mixed with annealed oligo 2/9, and annealed oligo 3/8 was mixed with annealed oligo 4/7. The resulting oligos were labeled oligo 1/10/2/9 and oligo 3/8/4/7. Next, oligo 3/8/4/7 was ligated to oligo 5/6. The resulting oligo 3/8/4/7/5/6 was then ligated to oligo 1/10/2/9 resulted in a full length variable region gene.

Alternatively, when groups of 12 oligos were used, the order of addition was: 1+12 = 1/12, 2+11=2/11, 3+10=3/10, 4+9=4/9, 5+8=5/8, 6+7=6/7, 1/12+2/11=1/12/2/11, 3/10+4/9=3/10/4/9, 5/8+6/7=5/8/6/7, 1/12/2/11+3/10/4/9 = 1/12/2/11/3/10/4/9, 1/12/2/11/3/10/4/9+5/8/6/7= full length variable region gene. The names of oligonucleotides used in construction of the engineered genes are listed in Table 5. The modified heavy chain variable region gene was denoted as 2CAVHCOL1. The modified light chain variable region gene was denoted as 2CAVLCOL1.

The resulting modified variable region genes were then purified by gel electrophoresis. To remove unligated excess of oligos and other incomplete DNA fragments, ligated product was run on 1% low melting agarose gel at constant 110 V for 2 hours. The major band containing full length DNA product was cut out and placed in a

sterile 1.5 ml centrifuge tube. To release the DNA from the agarose, the gel slice was digested with f3-Agrase I at 40°C for 3 hours. The DNA was recovered by precipitation with 0.3 M NaOAc and isopropanol at —20°C for 1 hour followed by centrifugation at 12,000 rpm for 15 minutes. The purified DNA pellet was resuspended in 50 µl of TE buffer. pH 8.0. The engineered variable region gene was then amplified by PCR. Specifically, 100 ng of the engineered variable region gene was mixed with 25mM dNTPs, 200 ng of primers and 5 U of high fidelity thermostable Pfu DNA polymerase in buffer. Resulting PCR product was analyzed on 1% agarose gel.

Each purified DNA corresponding to the engineered variable region gene was subsequently inserted into the pUC19 bacterial vector. pUC19, is a 2686 base pair, a high copy number *E. coli* plasmid vector containing a 54 base pair polylinker cloning site in lacZ and an Amp selection marker. In order to prepare the vector for insertion of the engineered variable region gene, 10µg of pUC19 was linearized with *Hinc II* (50 U) for 3 hours at 37°C resulting in a vector with blunt end sequence 5' GTC. To prevent self re-ligation, linear vector DNA was dephosphorylated with 25 U of calf intestine alkaline phosphatase (CIP) for 1 hour at 37°C. In order to insert the engineered variable region gene into the pUC19 vector, approximately 0.5 µg of dephosphorylated linear vector DNA was mixed with 3 µg of phosphorylated variable region gene in the presence of T4 DNA ligase (1000 U), and incubated at 16°C for 12 hours.

The bacterial vector containing the engineered variable region gene was then used to transform bacterial cells. Specifically, freshly prepared competent DH5-α cells, 50 µl, were mixed with 1 µg of pUC19 containing the engineered variable region gene and transferred to an electroporation cuvette (0.2 cm gap; Bio-Rad). Each cuvette was pulsed at 2.5 kV/200 ohm/25 µF in an electroporator (Bio-Rad Gene Pulser). Immediately thereafter, 1 ml of SOC media was added to each cuvette and cells were allowed to recover for 1 hour at 37°C in centrifuge tubes. An aliquot of cells from each transformation was removed, diluted 1:100, then 100 µl plated onto LB plates containing ampicillin (Amp 40 µg/ml). The plates were incubated at 37°C overnight due to the presence of the Amp marker. Only transformants containing pUC19 vector grew on LB/Amp plates.

A single transformant colony was picked and grown overnight in a 3 ml LB/Amp sterile glass tube with constant shaking at 37°C. The plasmid DNA was isolated using Easy Prep columns (Pharmacia Biotech.) and suspended in 100 µl of TE buffer, pH 7.5. To confirm the presence of gene insert in pUC19, 25 µl of plasmid DNA from each colony was digested with a restriction endonuclease for 1 hour at 37°C, and was analyzed on a 1% agarose gel. By this method plasmid DNA containing gene insert was resistant to enzyme cleavage due to loss of restriction site (5'..GTCGAC.. 3') and migrated as closed circular

(CC) DNA, while those plasmids without insert were cleaved and migrated as linear (L) double strand DNA fragment on gel.

In order to confirm correct gene sequences of the engineered variable region genes and to eliminate the possibility of unwanted mutations generated during the construction procedure, DNA sequencing was performed using M13/pUC reverse primer (5'AACAGCTATGACCATG 3') for the clones as well as PCR gene products using 5' end 20 base primer (5' GAATT CATGGCTTG GGTGTG 3') on automated ABI 377 DNA Sequencer. All clones were confirmed to contain correct sequences.

Table 5. Construction of gene encoding modified antibodies containing CDRs from Mab 31.1

	Oligo1	Oligo 2	Oligo 3	Oligo 4	Oligo 5	Oligo	Oligo 7	Oligo 8	Oligo 9	Oligo10
2CAVHC OLI	VHC1	VHC2	VHC3	VHC4	VHC5	VHC	VHC7	VHC8	VHC9	VHC10
2CAVLC OLI	VLC1	VLC2	VLC3	VLC4	VLCS	VLC	VLC7	VLC8	VLC9	VLC10

6.3. INSERTION OF THE ENGINEERED VARIABLE REGION GENE INTO A MAMMALIAN EXPRESSION VECTOR

A complete antibody light chain has both a variable region and a constant region. A complete antibody heavy chain contains a variable region, a constant region, and a hinge region. A modified variable region genes 2CAVHCOL1 or 2CAVLCOL1 were inserted into vectors containing appropriate constant regions. Engineered variable region genes lacking cysteine residues in the light chain, were inserted into the pMRRO10.1 vector Figure 6A. The pMRRO10.1 vector contained a human kappa light chain constant region. Insertion of the engineered light chain variable region into this vector gave a complete light chain sequence. Alternatively, the engineered variable region gene lacking cysteine residues in the heavy chain, were inserted into the pGAMMA1 vector Figure 6B. The pGAMMA1 vector contained human and IgG1 constant region and hinge region sequences. Insertion of the engineered heavy chain variable region gene into this vector gave a complete heavy chain sequence.

In order to engineer a mammalian vector comprising both heavy chain and light chain genes, the complete light chain sequence and complete heavy chain sequence were inserted into mammalian expression vector pNEPuDGV as shown in Figure 6C (Bebbington, C.R., 1991, In METHODS: A Companion to Methods in Enzymology, vol. 2, pp. 136-145). The resulting vector encoding both light chain and the heavy chain of the modified antibody.

6.4. EXPRESSION OF SYNTHETIC MODIFIED ANTIBODIES IN MAMMALIAN CELLS

To examine the production of assembled antibodies the mammalian expression vector was transfected into COS cells. COS cells (an African green monkey kidney cell line, CV-1, transformed with an origin-defective SV40 virus) were used for short-term transient expression of the synthetic antibodies because of their capacity to replicate circular plasmids containing an SV40 origin of replication to very high copy number. The antibody expression vector was transferred to COS7 cells (obtained from the American Type Culture Collection). The transfected cells were grown in Dulbecco's modified Eagle's Medium and transfected with the expression vectors using calcium precipitation (Sullivan et al., *FEBS Lett.* 285:120-123, 1991). The transfected cells were cultured for 72 hours after which supernatants were collected. Supernatants from transfected COS cells were assayed using ELISA method for assembled IgG. ELISA involves capture of the samples and standards onto a 96-well plate coated with an anti-human IgG Fc. Bound assembled IgG was detected with an anti-human Kappa chain linked to horse radish peroxidase (HRP) and the substrate tetramethylbenzidine (TMB). Color development was proportional to the amount of assembled antibody present in the sample.

6.5. MODIFIED ANTIBODY IMMUNOSPECIFICALLY BINDS TO HUMAN COLON CARCINOMA CELLS AND ANTIGENS PRODUCED BY THESE CELLS

The modified antibody was expressed and isolated as indicated in Section 6.4, *supra*. The binding capacity and specificity were then assayed using LS-174T cells WiDR cells (a human colon cancer cell line) and an antigen derived from these cells.

In order to examine the binding potency as well as specificity of MA31.1 binding, a dot blot analysis was performed (see Figure 9). Membrane preparations from LS-174T cells was applied to a nitrocellulose membrane using a Bio-Blot apparatus (Bio-Rad). The wells were blocked for non-specific binding using skim milk. Biotinylated antibody derived from Mab31.1 was incubated in all wells. Unlabelled antibody at concentrations of 0.003 to 20 nM was then applied to the nitrocellulose membrane and allowed to incubate. Unbound antibody was removed from the membrane by washing and a second antibody, alkaline phosphatase labeled antihuman IgG, was added. Finally, an alkaline phosphatase substrate was added which generated a dark purple precipitate, indicating the presence of bound labeled antibody. Figure 9 provides the results of the dot blot analysis. The figure demonstrated that the labeled antibody bound to the LS-174 T cells. Additionally, the unlabeled antibody competed with biotinylated antibody binding, indicating specificity of binding of the antibody derived from Mab31.1 to tumor cell antigens.

6.6. ANTI-IDIOTYPE RESPONSE

The effect on binding of modified antibody to LS-174T cells was examined in a competition binding assay. LS-174T cells are human colon carcinoma cells which express antigen recognized by the Mab31.1 antibody. Peptides containing the sequence of one of the CDRs of the Mab31.1 antibody were generated. These peptides, the modified antibody and the control antibody derived from Mab31.1 were administered to mice in order to generate antisera against the CDR regions of Mab31.1 and the antibodies. Blood samples from mice were drawn two weeks and four weeks following injection. Antisera from the immuized mice were used in binding competition assays presented in Figures 10A and B.

Antisera and biotinylated antibodies were assayed for their ability to bind LS-174T cells. As demonstrated in Figure 10A and B, antisera raised to the CDR3 and CDR4 peptides dramatically competed for control antibody (antibody derived from Mab31.1) binding to LS-174T cells. Additionally, antisera raised against CDR1 and CDR2 also significantly competed for control antibody binding to LS-174T cells. Additionally, antisera from nice injected with the 2CAVHCOL1 and 2CAVLCOL1 antibodies (*i.e.*, the modified antibodies having the cysteine to alanine change in the variable region) competed for binding with the biotinylated antibody derived from Mab31.1 better than antiserum from mice injected with the antibody derived from Mab31.1 (Figure 10B). This result indicates that administration of the antibodies having the cysteine to alanine change in the variable region elicit an anti-idiotype antibodies that recognize the colon carcinoma cell antigen better than antibodies with variable region intra-chain disulfide bonds.

Table 6. Biotin-Labeled Peptides Derived from CDR Sequences of Mab 31.1

Peptide ID	Sequence
COL311 L1	biotin-N-Thr-Ala-Lys-Ala-Ser-Gln-Ser-Val-Ser-Asn-Asp-Val-Ala
COL311 L2	biotin-N-Ile-Tyr-Tyr-Ala-Ser-Asn-Arg-Tyr-Thr
COL311 L3	biotin-N-Phe-Ala-Gln-Gln-Asp-Tyr-Ser-Ser-Pro-Leu-Thr
COL311 H1	biotin-N-Phe-Thr-Asn-Tyr-Gly-Met-Asn
COL311 H2	biotin-N-Ala-Gly-Trp-Ile-Asn-Thr-Tyr-Thr-Gly-Glu-Pro-Thr-Tyr-Ala-Asp-Asp-Phe-Lys-Gly
COL311 H3	biotin-N-Ala-Arg-Ala-Tyr-Tyr-Gly-Lys-Tyr-Phe-Asp-Tyr

7. EXAMPLE: SPERM ANTIGEN VACCINES

The example herein describes the construction of defined epitopes that replace the complementarity determining regions (CDR) of an antibody. Specifically, the

epitopes are derived from sperm antigens SP-10, LDH-C_4 or MSA-63. These constructs express an antibody, which, when injected into an appropriate host, induces an immune reaction that precipitates the formation of anti-idiotype antibodies that are active against the sperm antigens.

The strategy for producing the antibody containing a sperm cell epitope is outlined as follows: (1) a CDR is engineered to contain a nucleotide sequence encoding one or more epitopes from a sperm specific protein, (2) the engineered CDR is then cloned into a mammalian expression vector containing the appropriate heavy or light chain constant regions, (3) the vector is transfected into a cell that supports expression, proper folding and modification of functional antibodies, (4) the antibody is harvested from the supernatant and is confirmed for the epitope expression by standard assays (e.g. ELISA, western blot, etc.), and (5) the antibody is used as an immunogen in an appropriate host to generate anti-sperm antibodies, thereby inducing long lasting infertility.

7.1. CONSTRUCTION OF THE SPERM ANTIGEN VACCINE

The following describes the construction of a modified variable region gene containing at least one CDR that contains a sperm antigen epitope, *i.e.*, SP-10 or LDH-C_4 epitope and/or an MSA-63 epitope.

First, an epitope is chosen and defined so that oligonucleotides may be synthesized. In the following example, an SP-10 epitope from the sperm antigen SP-10 is used. SP-10 is a suitable epitope because it is expressed exclusively in sperm cells. It is also expressed on the surface of the membrane of the acrosome, thus, it is accessible to therapeutic antibodies. Other antibodies are produced that contain portions of the LDH-C_4 and MSA-63 antigens.

The nucleotide and protein sequences of the SP-10 epitope are:

GAA TTC CAG CCT TCA GGT GAA CAT GGC TCC GGT GAA CAG CCT TCT GGT GAG CAG GCC TCG GGT GAA CAG CCT TCA GGT GAG CAC GCT TCA GGG GAA CAG GCT TCA GGT GCA CCA ATT TCA AGC ACA TCT ACA GGC ACA ATA TTA AAT TGC TAC ACA TGT GCT TAT ATG AAT GAT CAA GGA AAA TGT CTT CGT GGA GAG GGA ACC TGC ATC ACT CAG AAT TC;

Gln Pro Ser Gly Glu His Gly Glu Gln Pro Ser Gly Glu Gln Ala Ser Gly Glu Gln Pro Ser gly Glu His Ala Ser Gly Glu Gln Ala Ser Gly Ala Gin Ile Ser Ser Thr Ser Thr Gly Thr Ile Leu Asn Cys Tyr Thr Cys Ala Tyr Met Asn Asp Gln Gly Lys Cys Leu Arg Gly Glu Gly Thr Cys Ile Thr Gln Asn.

The replacement of an antibody's CDR with another epitope is made easier by the fact that the variable region sequence of antibodies are relatively short, and are known. One is then able to synthetically generate a series of complementary oligonucleotides that, when annealed and ligated, reconstruct the entire coding region of

variable region portion of the gene. In this manner, the CDR is replaced with sequences of the epitope of interest, in this example, SP-10. The following is a list of the sequences of the oligonucleotides designed for cloning SP-10 epitopes into the CDR:

Oligo SP 1:
GAA TTC CAG CCT TCA GGT GAA CAT GGC TCC GGT GAA CAG CCT TCT GGT GAG CAG GCC TCG GGT GAA CAG CCT TAG,
Oligo SP 2:
GTG AGC ACG CTT CAG GGG AAC AGC CTT CAG GTG CAC CAA TTT CAA GCA CAT CTA CAG GCA CAA TAT TAA ATT GCT,
Oligo SP 3:
ACA CAT GTG CTT ATA TGA ATG ATC AAG GAA AAT GTC TTC GTG GAG AGG GAA CCT GCA TCA CTC AGA ATT C,
Oligo SP 3a(3Cys-> Ala):
ACA CAG CAG CTT ATA TGA ATG ATC AAG GAA AAG CAC TTC GTG GAG AGG GAA CCG CAA TCA CTC AGA ATT C,
Oligo SP 4:
GAA TTC TGA GTG ATG CAG GTT CCC TCT CCA CGA AGA CAT TTT CCT TGA TCA TTC ATA TAA GCA CAT GTG TAG CAA TTT A,
Oligo SP 4a (3Cys->Ala):
GAA TTC TGA GTG ATT GCG GTT CCC TCT CCA CGA AGT GCT TTT TGA TGA TCA TTC ATA TAA GCT GCT GTG TAG CAA TTT A,
Oligo SP 5:
ATA TTG TGC CTG TAG ATG TGC TTG AAA TTG GTG CAC CTG AAG CCT GTT CCC CTG AAG CGT GCT CAC CTG AAG GCT,
Oligo SP 6:
GTT CTC CCG AGG CCT GCT CAC CAG AAG GCT GTT CAC CGG AGC CAT GTT CAC CTG AAG GCT GGA ATT C.

Antibodies containing portions of the MSA-63 antigen are also described. To identify the optimal portion of the antigen to be introduced into the antibody, oligonucleotides encoding different portions of the antigen are synthesized.

Practically, the first two amino acid codons of the sperm cell specific epitope, MSA-63, an oligonucleotide encoding residues 143 and 144 (i.e. GTC GGC, *infra*), is cloned into the immunoglobulin CDR, using the methods described *infra*,. The MSA-63 DNA sequence encoding the epitope:

GTC GGC AGC CTC CGA AGC AGC CCG CTC CAG AGC CCG CTG CTC CGA CCG CTC GTC CAG AGC AGC CTC TGC TTG CTG TTC CTC TTG CTG CGA TAC AGC TGC GGC GAC GGC AGC TGC AGC CGA CGA TAC TGC GAC TTG ACG GTG TGC CGG CGA ATG TAC TTG CTG CTG CGA TTC ACG GAC CCG CCG CTC CCG CAG ACG TGC TGC GTC TTG AGC

The MSA-63 protein sequence epitope encoded by the nucleic acid sequence above, which starts at amino acid 143 and ends at 233.

Gln Pro Ser Glu Ala Ser Ser Gly Glu Val Ser Gly Asp Glu Ala Gly Glu Gln Val Ser Ser Glu Thr Asn Asp Lys Glu Asn Asp Ala Met Ser Thr Pro Leu Pro Ser Thr Ser Ala Ala Ile

Thr Leu Asn Cys His Thr Cys Ala Tyr Met Asn Asp Asp Ala Lys Cys Leu Arg Gly Glu Gly Val Cys Thr Thr Gln Asn Ser

For the second two amino acid codons, an oligonucleotide encoding residues 144 and 145 is utilized (*i.e.*, GGC AGC). For the third, 145 and 146 and so on until the entire epitope is synthesized and inserted into the CDR, two amino acids at a time. For peptides three amino acids in length, an oligonucleotide encoding residues 143 to 146 is synthesized. The second oligonucleotide synthesized encodes residues 146 to 148. The third encodes residues 148 to 150, and this continues until the entire epitope is covered in this fashion. The next oligonucleotide that is synthesized is four amino acid codons in length. It begins with residues 143 to 146, its second segment is equivalent to residues 145 to 148, its third segment corresponds to residues 147 to 150, and so on until the entire epitope is transitioned in this fashion. The next oligonucleotide synthesized contains five amino acid codons with two overlapping with the previous. For example, the first oligonucleotide encodes residues 143 to 147, and the second residues 146 to 150. This pattern continues until the entire epitope has been transitioned. The next construct encoding an epitope uses nucleotides for six amino acid codons with two overlapping with the previous codons as described *infra*.

The epitopes thereafter contain peptides of seven residues with three overlapping. The pattern of adding one amino acid to each small peptide and increasing the overlap by one codon continues until an overlap of five is reached and then the small peptides are synthesized adding one codon each time until the full length of the epitope is encoded in the CDR. The overlap is never bigger than five amino acid codons although the entire peptide is lengthened by one amino acid in each new combination.

In a specific example, oligomers have been designed which scan the entire length of the MSA-63 epitope and encode 15 amino acids. Each oligo overlaps with the previous one for the equivalent of five amino acids. MSA-63 oligos encoding 15 amino acids, with overlap of five amino acids each:

MSA1: GTC GGC AGC CTC CGA AGC AGC CCG CTC CAG AGC CCG CTG CTC CGA

MSA2: AGC CCG CTG CTC CGA CCG CTC GTC CAG AGC AGC CTC TGC TTG CTG

MSA3: AGC CTC TGC TTG CTG TTC CTC TTG CTG CGA TAC AGC TGC GGC GAC

MSA4: TAC AGC TGC GGC GAC GGC AGC TGC AGC CGA CGA TAC TGC GAC TTG

MSA5: CGA TAC TGC GAC TTG ACG GTG TGC ACG CGA ATG TAC TTG CTG CTG

MSA6: ATG TAC TTG CTG CTG CGA TTC ACG GAC GCG CCG CTC CCG CAG ACG

MSA7: CGA TTC ACG GAC GCG CCG CTC CCG CAG ACG TGC TGC GTC TTG AGC

Antibodies in which a portion of the MSA-63 antigen has been inserted into CDR1, *i.e.*, where residues Lys24 through Ala34 of the consensus contraceptive light chain, the sequence of which is in Figure 15, are replaced with the sequence Gln-Pro-Ser-Glu-Ala-Ser-Ser-Gly-Glu-Val-Ser-Gly-Asp-Glu-Ala-Gly-Glu. The antibody, MSA1, can be constructed using the oligonucleotides provided in Figure 11 in the scheme provided in Figure 8 and described below, where the identities of oligonucleotides 1-12 are indicated in Table 7. The antibody MSA1VAC can also be constructed using the oligos of Figure 11 by the scheme of Figure 8, as indicated in Table 7. MSA1VAC is the same as MSA1 except that the cysteine at position 23 of the light chain variable region has been replaced with alanine. These light chains can be expressed with the heavy chain consensus sequence CONVH1, the sequence of which is provided in Figure 7B, and the construction of which can be accomplished with the oligonucleotides as indicated in Table 4. These single stranded oligonucleotides sequences are annealed to create cohesive double stranded DNA fragments suitable for ligation as diagramed in Figure 8, along with oligonucleotides encoding the remainder of the consensus variable region, to construct the variable region gene. For the MSA-63 containing variable regions MSA1 and MSA1VAC the oligonucleotides corresponding to oligonucleotides 1 to 10, or 1 to 12, of Figure 8 are provided in Table 7, and the sequences of these oligonucleotides are provided in Figure 11. Specifically, oligonucleotides of about 70 bases in length corresponding to the sequences of interest with 20 base overlapping regions are synthesized (GenoSys Biotech Inc.). Each oligonucleotide is 5' phosphorylated as follows: 25µl of each oligo is incubated for one hour in the presence of T_4 polynucleotide kinase and 50 mM ATP in appropriate buffer at 37°C. The enzyme is heat killed and the reaction stopped by heating for ten minutes at 70°C followed by ethanol precipitation with sodium acetate. The oligos are then resuspended in TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA).

Complementary oligonucleotides (oligo 1 + oligo 10, oligo 2 + oligo 9, oligo 3 + oligo 8, oligo 4 + oligo 7, and oligo 5 + oligo 6) were then mixed in a sterile microcentrifuge tube and annealed by heating the tube in a water bath at 65°C for 5 minutes followed by cooling at room temperature for 30 minutes. Annealing results in double stranded DNA with cohesive ends. The cohesive double stranded DNA fragments are ligated into longer strands (Figure 8, Steps 2-4), until the engineered variable region gene was assembled. Specifically, cohesive double stranded DNA fragments are ligated in the presence of T_4 DNA ligase, ligase buffer and 10 mM ATP for two hours in a water bath maintained at 16°C. Annealed oligo 1/10 is mixed with annealed oligo 2/9, and annealed oligo 3/8 is mixed with annealed oligo 4/7. The resulting oligos are 1/10/2/9 and 3/8/4/7. Next, oligo 3/8/4/7 is ligated to oligo 5/6. The resulting oligo 3/8/4/7/5/6 is then ligated to

oligo 1/10/2/9 resulting in a full length variable region gene. Alternatively, when 12 oligos are used, the order of addition is 1+12=1/12, 2+11=2/11, 3+10=3/10, 4+9=4/9, 5+8=5/8, 6+7=6/7, 1/12+2/11=1/12/2/11, 3/10+4/9=3/10/4/9, 5/8+6/7=5/8/6/7, 1/12/2/11+3/10/4/9=1/12/2/11/3/10/4/9, 1/12/2/11/3/10/4/9+5/8/6/7=1/12/2/11/3/10/4/9/5/8/6/7, which is the full length modified variable region gene. The names of oligonucleotides used for construction are listed in Table 7 and Figures 9, , 11, 12C, or 13C.

Using this method, variable region sequences in which an alanine has been substituted for a cysteine that forms an intrachain disulfide bond can be constructed using oligonucleotides introducing this change. For example, in constructing the antibody contains the SP-10 portion, oligos SP 3a and SP 4a could be used instead of oligo SP3 or SP4.

The modified variable region DNA fragment is then cloned into a shuttle vector (e.g. pUC19, *infra*) for sequence analysis and upon sequence confirmation, cloned into an expression vector. After running the DNA for two hours at 110 volts in a 1% low melting agarose gel, DNA fragments are visualized by ethidium bromide staining and gel slices are cut out and placed in a sterile microfuge tube. Gel purification removes excess free oligomers that may interfere with future ligations. The DNA is eluted from the agarose by addition with β-Agrase I at 40°C for three hours. DNA is precipitated using 0.3 M sodium acetate and isopropanol at -20°C for one hour, followed by centrifugation at high speed in a microcentrifuge for ten minutes. Isopropanol is aspirated and the pellet is washed once with 70% ethanol, the sample is spun again and the ethanol is aspirated and the pellet air dried. The DNA pellet is quantitated by running a small fraction of the resuspended pellet (i.e. 1/10th) on a gel and visually comparing to known DNA standards, or measuring the absorbance of UV light at 260 nM. If the quantity of DNA is to limiting for cloning at this point, it can be amplified by PCR techniques well known to those skilled in the art.

7.2 LIGATION OF THE MODIFIED CDR INTO PUC19

Purified DNA corresponding to the engineered variable region gene is subsequently inserted into the pUC19 vector by ligation. The pUC19 vector is a 2686 base

Table 7

	Oligo 1	Oligo 2	Oligo 3	Oligo 4	Oligo 5	Oligo 6	Oligo 7	Oligo 8	Oligo 9	Oligo 10	Oligo 11	Oligo 12
MSA 1	LDR	DSABL-1	MSAL-CDR1-1	HMVL3	HMVL4	HMVL5	HMVL6	HMVL7	HMVL8	MSAL-CDR1-1c	DSABL-1c	ANTIL DR
MSA1VAC	LDR	DSABL-1	MSALVAC-CDR1-1	HMVL3	HMVL4	HMVL5	HMVL6	HMVL7	HMVL8	MSALVAC-CDR1-1	DSABL-1c	ANTIL DR
ConVH1	BKHC1	BKHC2	BKHC3	BKHC4	BKHC5	BKHC6	BKHC7	BKHC8	BKHC9	BKHC10		

pair, high copy number *E. coli* plasmid containing a 54 base pair polylinker cloning site in the middle of the lacZ gene. The pUC19 vector also contains an ampicillin resistance marker for selection of bacteria containing the plasmid. The pUC19 is digested with the restriction enzyme *Hinc II* (10 μg plasmid in 50 units enzyme). The resulting blunt ends are dephosphorylated with calf intestinal phosphatase (CIP, 2 units in alkaline buffer, 30 minutes at 37°C), to prevent recircularization during the ligation step. The phosphatase is removed by extraction with phenol and chloroform, followed by precipitation with sodium acetate and ethanol on ice for 1 hour. The precipitated DNA is pelletted by high speed centrifugation and the ethanol is removed by aspiration, followed by a washing step with 70% ethanol to remove excess salts. The DNA pellet is air dried to completely remove any ethanol. The digested, phosphatased vector is then resuspended in TE buffer to 0.5 μg/μl. Approximately 0.1-0.5 μg of vector is incubated with a ten fold molar excess of the constructed variable region containing the sperm cell epitope in the CDR (modified variable region) with T_4 ligase (1000 units) in appropriate buffer and incubated at 16°C for 12 hours.

7.3 BACTERIAL TRANSFORMATION

The ligation mixture containing the engineered variable region gene cloned into pUC19, is transformed into competent bacterial cells. Specifically, 50 μl of freshly prepared competent DH5-α cells are mixed with the ligation mixture of pUC19 and modified variable region DNA and transferred to an electroporation cuvette (0.2 cm gap; Bio-Rad). Each cuvette is pulsed at 2.5 kV/200 ohm/25 μF in an electroporator (Bio-Rad Gene Pulser). Immediately thereafter, 1 ml of SOC media is added to each cuvette and cells are allowed to recover for 1 hour at 37°C in centrifuge tubes. An aliquot of cells from each transformation is removed, diluted 1:100, then 100 μl is plated onto LB plates with ampicillin (Amp 40 μg/ml). The plates are then incubated at 37°C overnight and only cells containing a plasmid grow.

The plasmid DNA is analyzed after isolation from single colonies picked by sterile toothpick and grown up overnight in 3 ml LB/Amp in a sterile glass test tube, with constant shaking at 37°C. The plasmid DNA is isolated using Easy Prep columns (Pharmacia Biotech) and suspended in 100 μl of TE buffer. To confirm the presence of insert, isolated plasmid DNA is digested with *Hinc II* and the digestion product is analyzed by 1.2% agarose gel electrophoresis in Tris-Acetate EDTA buffer (TAE). DNA is stained in the gel with ethidium bromide and visualized under UV light. The colonies that correspond to plasmids with insert are selected for further analysis.

7.4 DNA SEQUENCING

DNA sequencing is performed to verify the accuracy of the sequence in the cloned fragment. Sequencing across the pUC19 polylinker is done using the M13/pUC universal forward and universal reverse primers using the Sanger dideoxy chain termination procedure. The M13/pUC universal primers are readily found in biotechnology supply catalogues. Sequencing is performed on the ABI377 DNA sequencer, and sequence comparison is performed using standard computer alignment programs or visual inspection.

7.5 CLONING INTO THE V_H AND V_L CHAIN CONSTRUCTS

Once the sequence of the modified CDR has been confirmed, it is cut out of the pUC19 plasmid and ligated into either the heavy or light chain antibody expression vectors pMRRO10.1 or pGAMMA1, respectively (See Figures 6A and B). Alternatively, both the heavy and light chain genes are expressed on the same plasmid, and the modified CDR is ligated into either the heavy or light chain variable region as appropriate.

A complete antibody light chain has both a variable region and a constant region. A complete antibody heavy chain contains a variable region, a constant region, and a hinge region. The synthetic variable region genes of the invention are inserted into vectors containing appropriate constant regions. Engineered variable region genes with the sperm antigen epitope sequences are cloned into the pMRRO10.1 vector. The pMRRO10.1 vector contains a human kappa light chain constant region. Insertion of the engineered light chain variable region into this vector gives a complete light chain sequence. Alternatively, the engineered variable region gene with the sperm antigen sequence, of the heavy chain is inserted into the pGAMMA1 vector. The pGAMMA1 vector contains human and IgG1 constant region and hinge region sequences. Insertion of the engineered heavy chain variable region gene into this vector gave a complete heavey chain sequence.

In order to engineer a mammalian vector comprising both heavy chain and light chain genes, the complete light chain sequence and heavy chain sequence were inserted into a mammalian expression vector pNEPuDGV (Figure 6C; Bebbington, C., 1991, In METHODS: A Companion to Methods in Enzymology, 2:136-145). The resulting vector encodes both light chain and the heavy chain of the antibody.

7.6 TRANSFECTION OF EUKARYOTIC CELLS

The antibody expression plasmid, pNEPuDGV, is then transfected into a suitable host cell for expression of the antibody of interest. COS-7 (an African green monkey kidney cell line, CV-1, transformed with an origin defective SV40 virus), 293, or CHO cells are capable of being transfected and support expression of foreign proteins.

Transfection is performed by standard calcium phosphate precipitation (Sullivan et al., 1991, FEBS Lett. 285:120-123). Alternatively, cells may be transfected using lipid vesicles or electroporation. Transient or stable transfections are suitable depending on how much protein is expressed and harvested.

7.7 EXPRESSION AND PROTEIN ANALYSIS

Transfected cell supernatants are collected and analyzed for proper expression of anti-idiotype antibodies. The antibodies are purified away from cell debris and growth media serum and also concentrated from the supernatant by binding the antibody Fc domain to a protein A or protein G column. The antibody is eluted from the column by low pH glycine and dialyzed against BSA and Tris buffer.

7.8 IN VIVO ANALYSIS OF ANTI-IDIOTYPE EFFICACY

To test the ability of the antibody to elicit an immune response or for a contraceptive effect, the antibody is injected into a mouse at a pharmaceutically significant dose range and serum samples are taken from the mice. The production of anti-idiotype antibodies is confirmed by harvesting peripheral blood serum and performing ELISAs with the sperm antigen (or sperm), or western blots using the sperm antigen (or sperm) as target and the vaccinated mouse serum as probe.

ELISA involves capture of the samples and standards onto a 96 well plate coated with an anti-epitope antibody. Bound antibody is detected with a secondary antibody crosslinked to horse radish peroxidase (HRP) and the substrate tetramethylbenzidine (TMB) and specific to the kappa or lambda light chain of the mouse. Alternatively, western blots are performed using the anti-idiotype as the target and probing it with anti-epitope antibodies.

Confirmation of production of anti-idiotypes in the mice is then followed by *in vivo* analysis to determine whether the mice are capable of conception. Control mice and test mice are mated in statistically significant groups and the number of pregnancies are monitored. Effective immunocontraceptive therapy will result in a significant reduction in the number of pregnancies.

Additionally, the induction of effective quantities of anti-idiotype anti-bodies is also assayed for prevention of *in vitro* fertilization. Donor sperm is mixed in vitro with donor eggs in the presence or absence of test serum or negative control serum. The failure of sperm to fertilize the egg when test serum is added is a positive indication that the vaccine is effective.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. A vaccine composition comprising an amount of a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said first immunoglobulin molecule comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule having at least one complementarity determining region (CDR) that has a portion of an antigen of a cell or protein involved in reproductive function, said one or more amino acid substitutions being the substitution of one or more amino acid residues that do not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule; and a pharmaceutically acceptable carrier.

2. The vaccine composition according to claim 1, wherein said antigen is a sperm antigen.

3. The vaccine composition according to claim 2, wherein said sperm antigen is SP-10, MSA-63 or LDH-C4.

4. The vaccine composition according to claim 1, wherein said antigen is selected from the group consisting of gonadotropin-releasing hormone, a gonadotropin, prostaglandin F2 alpha, oxytocin, gonadotropin receptors, SP-17, PH-20, FA-1, FA-2, PH-30, RSA, HAS-63, ZP1, ZP2, and ZP3.

5. The vaccine composition according to claim 1, wherein a first CDR contains a portion of an antigen of a cell or protein associated with reproductive function and a second CDR contains a portion of an antigen of a cell or protein associated with reproductive function.

6. The vaccine composition according to claim 5, wherein said first CDR contains a portion of SP-10 antigen, and said second CDR contains a portion of LDH-C4.

7. The vaccine composition according to claim 1, wherein said variable region is a light chain variable region and said amino acid residue that does not have sulfhydryl group is at a position corresponding to position 23 or 88 in said light chain variable regionof said second immunoglobulin molecule.

8. The vaccine composition according to claim 1, wherein said variable region is a heavy chain variable region and said amino acid residue that does not have a sulfhydryl group is at a position corresponding to position 22 or 92 in said heavy chain variable region of said second immunoglobulin molecule.

9. The vaccine composition according to claim 1, 7 or 8, wherein said amino acid residue is alanine.

10. The vaccine composition according to claim 1, in which said first immunoglobulin molecule is of a type selected from the group consisting of IgG, IgE, IgM, IgD and IgA.

11. A vaccine composition comprising an amount of a fragment of a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said first immunoglobulin molecule comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule having at least one complementarity determining region (CDR) that has a portion of an antigen of a cell or protein involved in reproductive function, said one or more amino acid substitutions being the substitution of one or more amino acid residues that do not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule; and a pharmaceutically acceptable carrier.

12. The vaccine composition according to claim 11, wherein said antigen is a sperm antigen.

13. The vaccine composition according to claim 12, wherein said sperm antigen is SP-10, MSA-63 or LDH-C4.

14. The vaccine composition according to claim 11, wherein said antigen is selected from the group consisting of gonadotropin-releasing hormone, a gonadotropin, prostaglandin F2 alpha, oxytocin, gonadotropin receptors, SP-17, PH-20, FA-1, FA-2, PH-30, RSA, HAS-63, ZP1, ZP2, and ZP3.

15. The vaccine composition according to claim 11, wherein a first CDR contains a portion of an antigen of a cell or protein associated with reproductive function

and a second CDR contains a portion of an antigen of a cell or protein associated with reproductive function.

16. The vaccine composition according to claim 15, wherein said first CDR contains a portion of SP-10 antigen, and said second CDR contains a portion of LDH-C4.

17. The vaccine composition according to claim 11, wherein said variable region is a light chain variable region and said amino acid residue that does not have sulfhydryl group is at a position corresponding to position 23 or 88 in said light chain variable regionof said second immunoglobulin molecule.

18. The vaccine composition according to claim 11, wherein said variable region is a heavy chain variable region and said amino acid residue that does not have a sulfhydryl group is at a position corresponding to position 22 or 92 in said heavy chain variable region of said second immunoglobulin molecule.

19. The vaccine composition according to claim 11, 17 or 18, wherein said amino acid residue is alanine.

20. The vaccine composition according to claim 11, in which said first immunoglobulin molecule is of a type selected from the group consisting of IgG, IgE, IgM, IgD and IgA.

21. A method of contraception in a subject comprising administering to said subject an amount of a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said first immunoglobulin molecule comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule having at least one complementarity determining region (CDR) that has a portion of an antigen of a cell or protein involved in reproductive function, said one or more amino acid substitutions being the substitution of one or more amino acid residues that do not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule.

22. The method according to claim 21 which further comprises isolating an antibody from said subject, said antibody recognizing the idiotype of said second immunoglobulin molecule and administering said antibody to a second subject.

23. The method according to claim 21, wherein said antigen is a sperm antigen.

24. The method according to claim 23, wherein said sperm antigen is SP-10, MSA-63 or LDH-C4.

25. The method according to claim 21, wherein said antigen is selected from the group consisting of gonadotropin-releasing hormone, a gonadotropin, prostaglandin F2 alpha, oxytocin, gonadotropin receptors, SP-17, PH-20, FA-1, FA-2, PH-30, RSA, HAS-63, ZP1, ZP2, and ZP3.

26. The method according to claim 21, wherein a first CDR contains a portion of an antigen of a cell or protein associated with reproductive function and a second CDR contains a portion of an antigen of a cell or protein associated with reproductive function.

27. The method according to claim 26, wherein said first CDR contains a portion of SP-10 antigen, and said second CDR contains a portion of LDH-C4.

28. The method according to claim 21, wherein said variable region is a light chain variable region and said amino acid residue that does not have sulfhydryl group is at a position corresponding to position 23 or 88 in said light chain variable regionof said second immunoglobulin molecule.

29. The method according to claim 21, wherein said variable region is a heavy chain variable region and said amino acid residue that does not have a sulfhydryl group is at a position corresponding to position 22 or 92 in said heavy chain variable region of said second immunoglobulin molecule.

30. The method according to claim 21, 28 or 29, wherein said amino acid residue is alanine.

31. The method according to claim 21, in which said first immunoglobulin molecule is of a type selected from the group consisting of IgG, IgE, IgM, IgD and IgA.

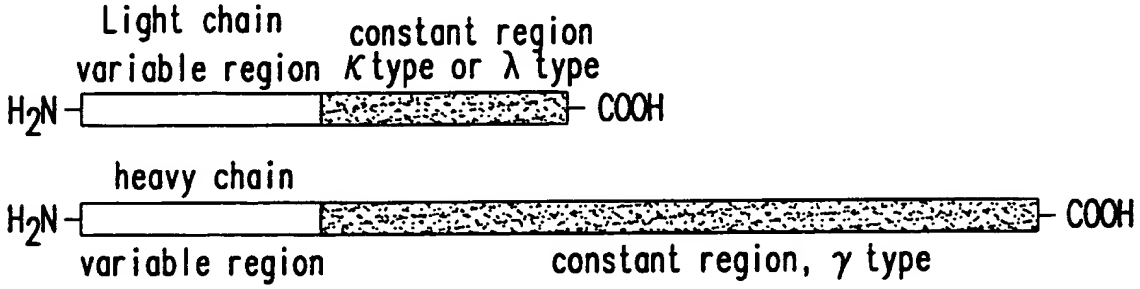
Light chain
variable region
constant region
κ type or λ type
H2N
COOH
heavy chain
H2N
COOH
variable region
constant region, γ type

FIG. 1

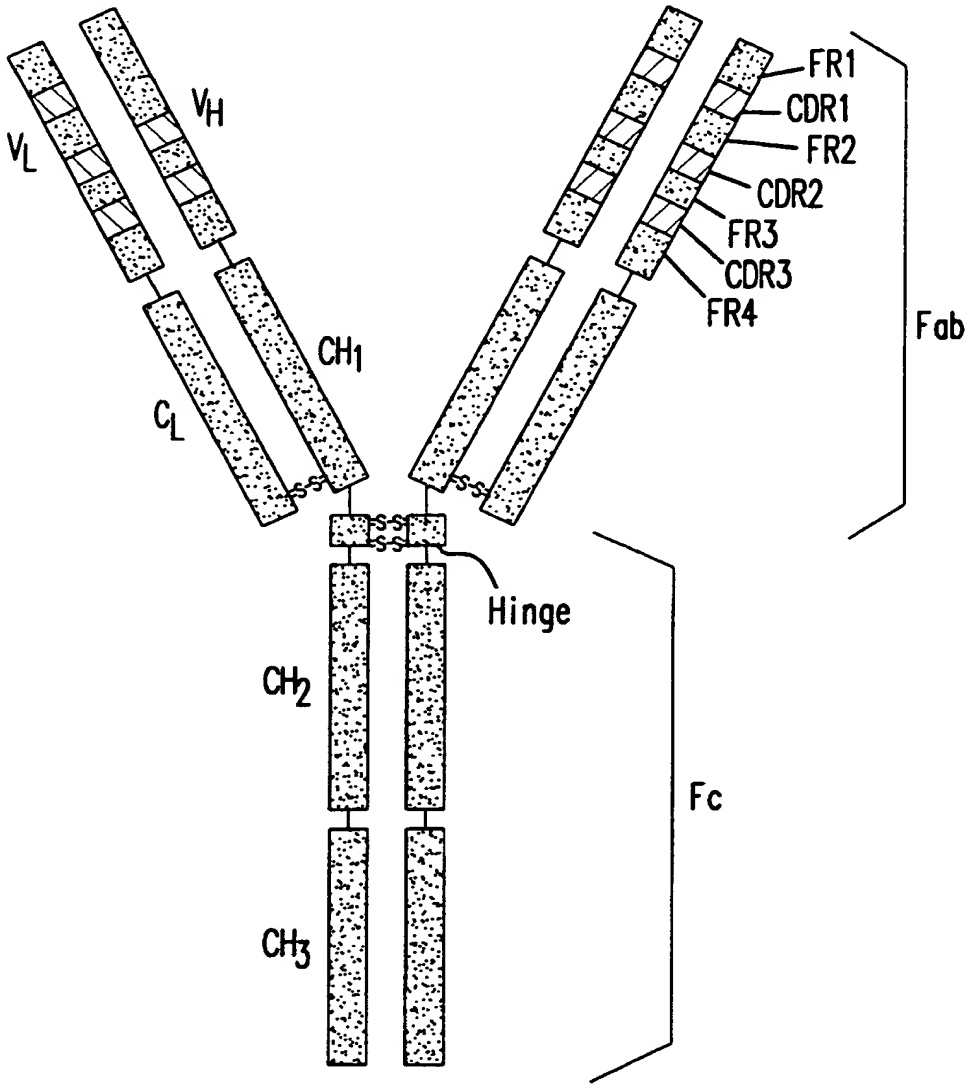

FIG.2

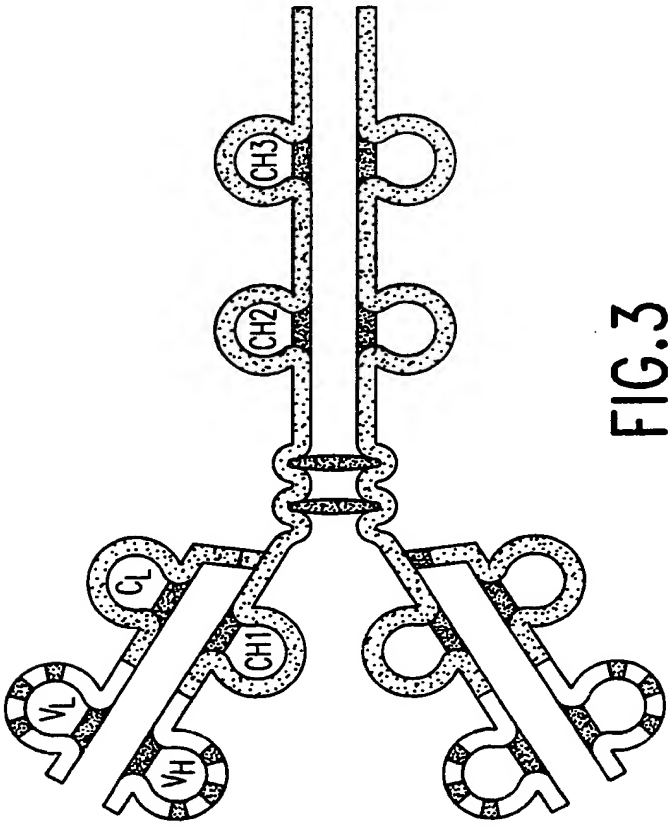

FIG.3

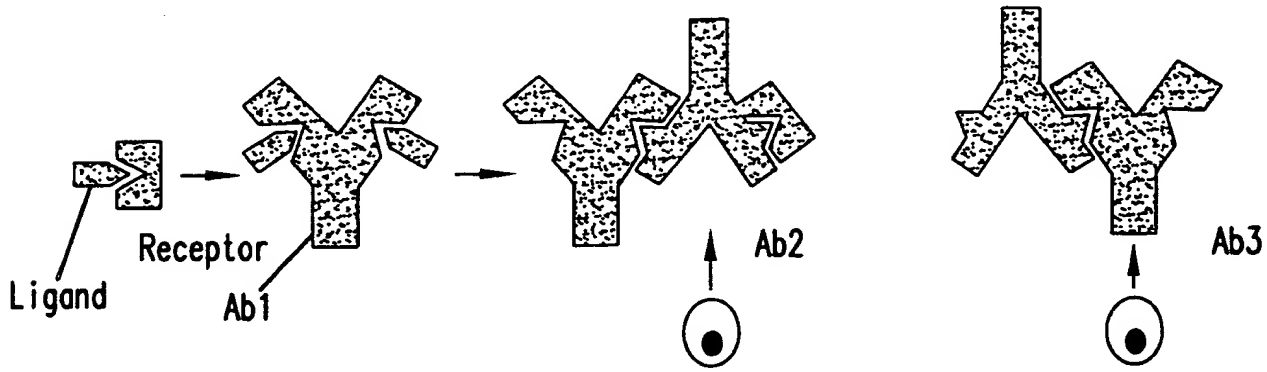

FIG.4

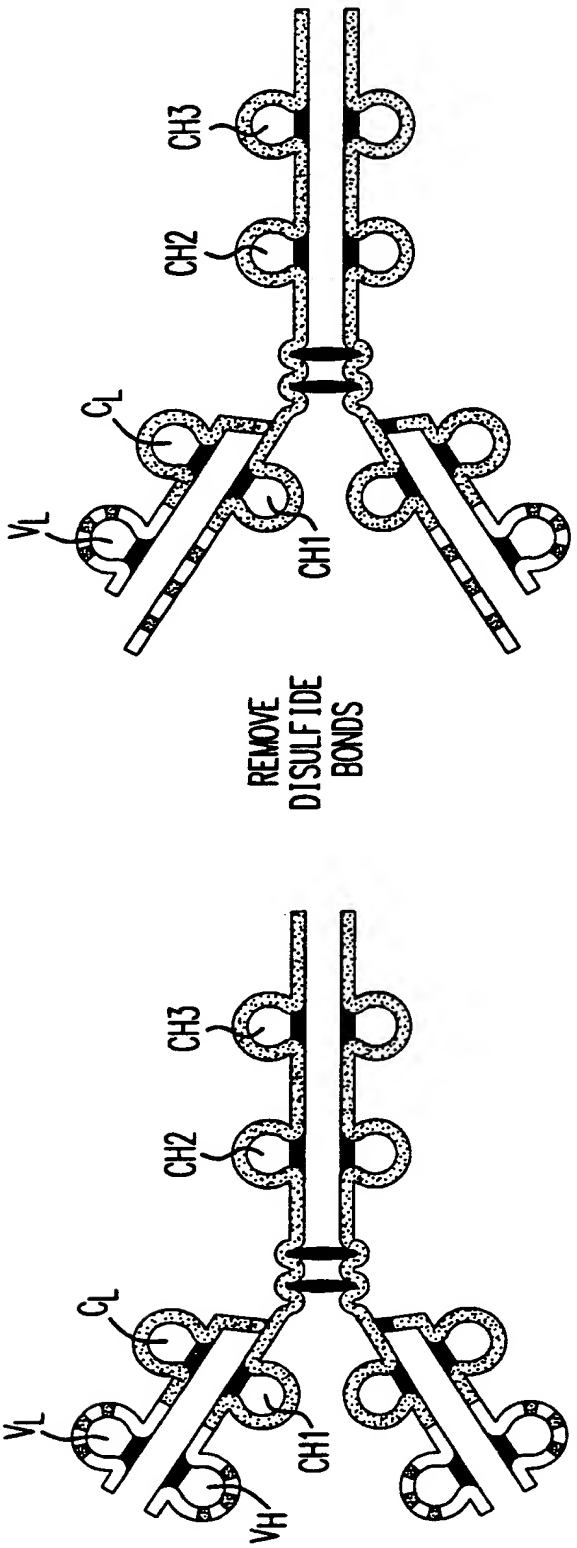

FIG.5

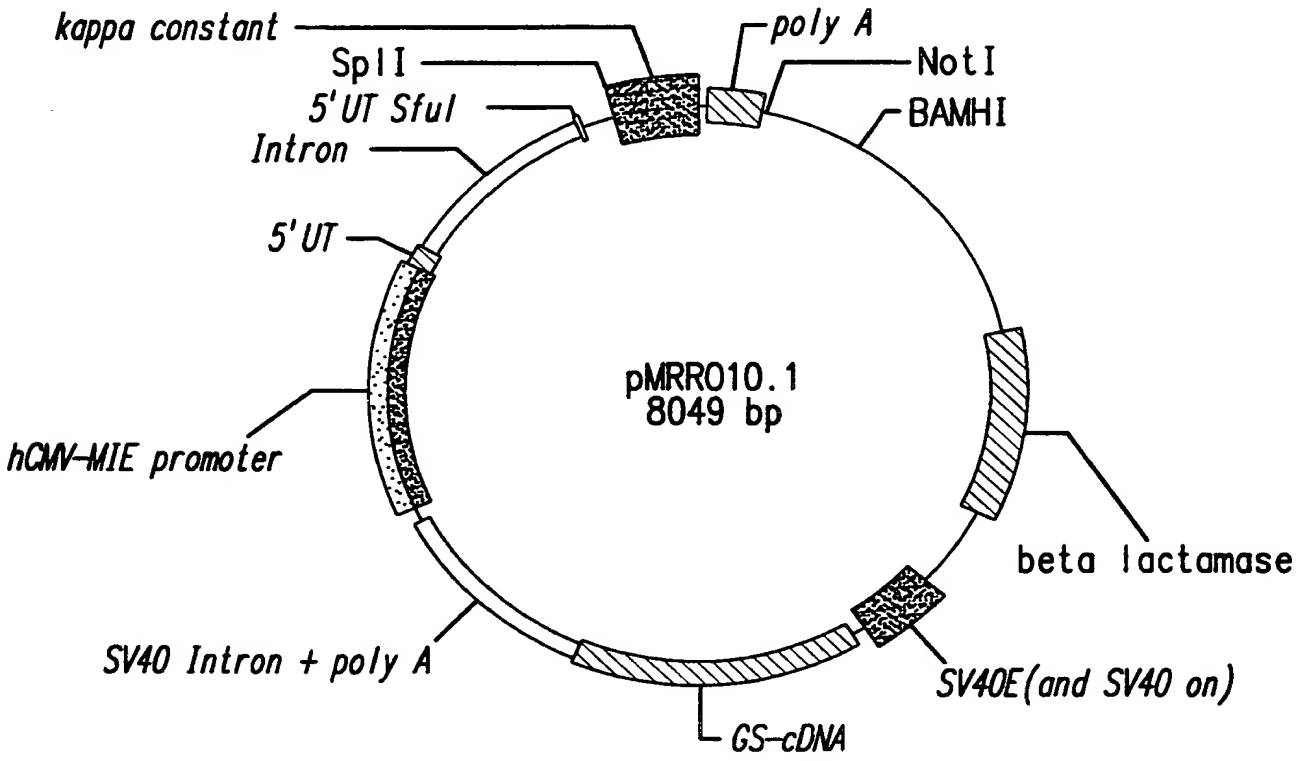
kappa constant
SpI I
5'UT Sful
Intron
5'UT
hCMV-MIE promoter
SV40 Intron + poly A
GS-cDNA
SV40E(and SV40 on)
beta lactamase
BAMHI
NotI
poly A
pMRR010.1
8049 bp

FIG.6A

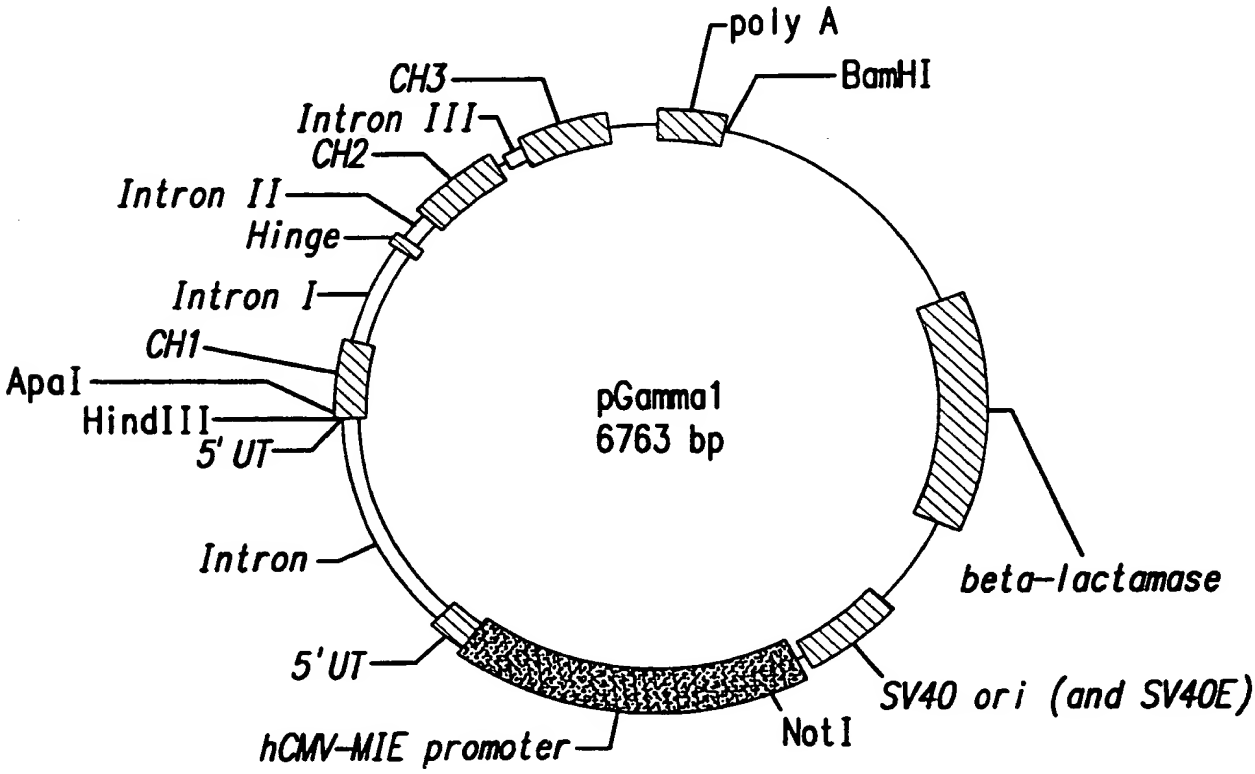
poly A
BamHI
CH3
Intron III
CH2
Intron II
Hinge
Intron I
CH1
ApaI
HindIII
5' UT
Intron
5'UT
hCMV-MIE promoter
NotI
SV40 ori (and SV40E)
beta-lactamase
pGamma1
6763 bp

FIG.6B

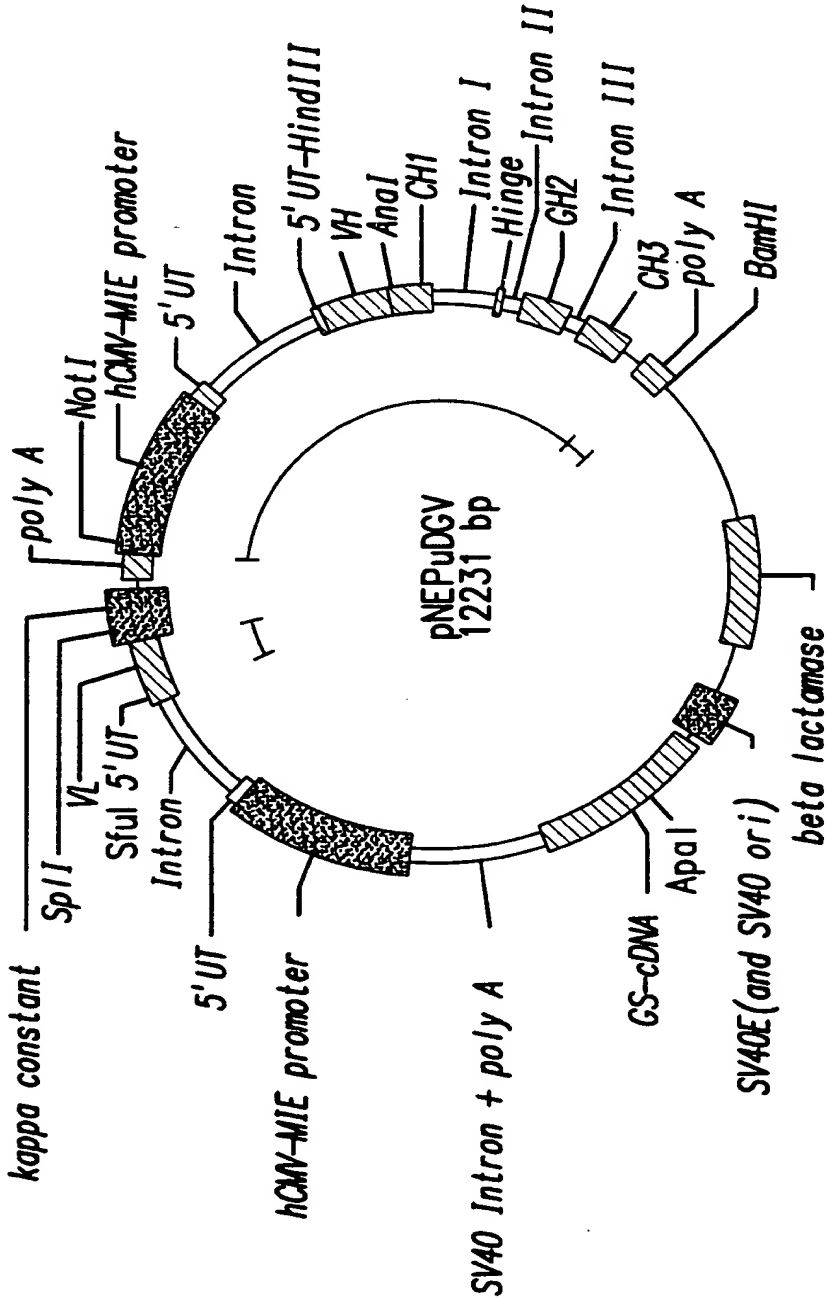
pNEPuDGV
12231 bp
poly A
NotI
hCMV-MIE promoter
5' UT
Intron
5' UT-HindIII
VH
AnaI
CH1
Intron I
Hinge
Intron II
CH2
Intron III
CH3
poly A
BamHI
beta lactamase
SV40E(and SV40 ori)
ApaI
GS-cDNA
SV40 Intron + poly A
hCMV-MIE promoter
5' UT
Intron
SfuI 5' UT
VL
SpII
kappa constant

FIG.6C

ConVL1

EcoR1
GAA TTC

6

-19 (Leader) -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
63

V_L:

1 10 20
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
Thr
GAT ATC CAA ATG ACA CAA AGT CCT AGT AGT TTG AGT GCT AGT GTG GGA GAT CGG GTG
ACA 123

21 30 40
Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys
Pro
ATC ACA TGT CGG GCT AGT CAA AGT ATC AGT AAC TGT TTG GCT TGG TAT CAA CAA AAG
CCT 183

41 50 60
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro
Ser
GGA AAG GCT CCT AAG TTG TTG ATC TAT GCT GCT AGT AGT TTG GAG AGT GGA GTG CCT
AGT 243

61 70 80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Arg Phe Thr Leu Thr Ile Ser Ser Leu Gln
Pro
CGG TTC AGT GGA AGT GGA AGT GGA ACA CGG TTC ACC TTG ACC ATC AGT AGT TTG CAA
CCT 303

81 90 100
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Leu Pro Trp Thr Phe Gly
Gln
GAG GAT TTC GCT ACC TAT TAT TGT CAA CAA TAT AAC AGT TTG CCT TGG ACC TTC GGA
CAA 363

101
Gly Thr Lys Val Glu Ile Lys
GGA ACC AAG GTG GAG ATC AAG GAA TTC 390
Eco R1

FIG.7A

ConVH1

EcoR1
GAA TTC 6

-19 (Leader) -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala
Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC
CAA AGT GCC CAA GCA 63

V_L:
1 10 20
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro
Gly Ala Ser Val Lys Val
CAG GTT CAG CTG GTG CAG TCT GGC GCT GAG GTG AAG AAG CCT
GGC GCT TCT GTG AAG GTG 123

21 30 35A 35B
40
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Ala Ile
Ser Trp Asn Trp Val Arg Gln Ala
TCT TGC AAG GCT TCT GGC TAC ACA TTC ACA TCT TAC GCT ATA
TCT TGG AAT TGG GTG AGG CAG GCT 189

41 50 60
Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Gly Asn
Gly Asp Thr Asn Tyr Ala
CCT GGC CAG GGC CTG GAG TGG ATG GGC TGG ATA AAT GGA AAT
GGA GAT ACA AAT TAC GCC 249

61 70 80
Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser
Thr Ser Thr Ala Tyr Met
CAG AAG TTC CAG GGA AGG GTG ACT ATA ACT GCT GAT ACT TCT
ACT TCT ACT GCT TAC ATG 309

81 82A 82B 82C 90
100
Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr
Cys Ala Arg Ala Pro Gly Tyr Gly Ser
GAG CTG TCT TCT CTG AGG TCT GAG GAT ACT GCT GTT TAC TAC
TGC GCT AGG GCT CCT GGC TAC GGC TCT 378

101 110
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
GAT TAT TGG GGA CAG GGA ACA CTG GTT ACA GTT TCT TCT GAA TTC
423

FIG.7B

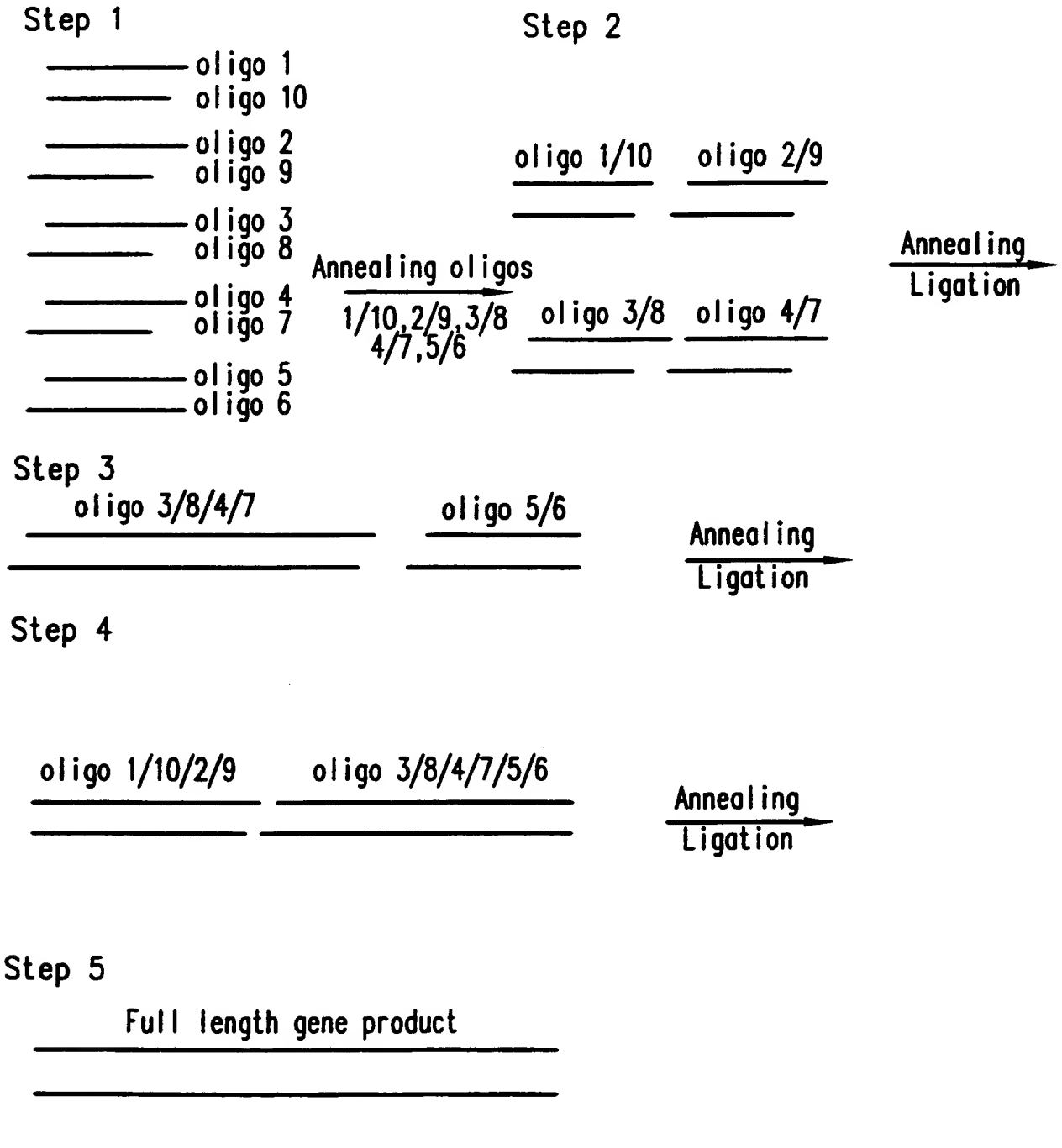
Step 1
oligo 1
oligo 10
oligo 2
oligo 9
oligo 3
oligo 8
oligo 4
oligo 7
oligo 5
oligo 6
Annealing oligos
1/10,2/9,3/8
4/7,5/6
Step 2
oligo 1/10
oligo 2/9
oligo 3/8
oligo 4/7
Annealing
Ligation
Step 3
oligo 3/8/4/7
oligo 5/6
Annealing
Ligation
Step 4
oligo 1/10/2/9
oligo 3/8/4/7/5/6
Annealing
Ligation
Step 5
Full length gene product

FIG.8

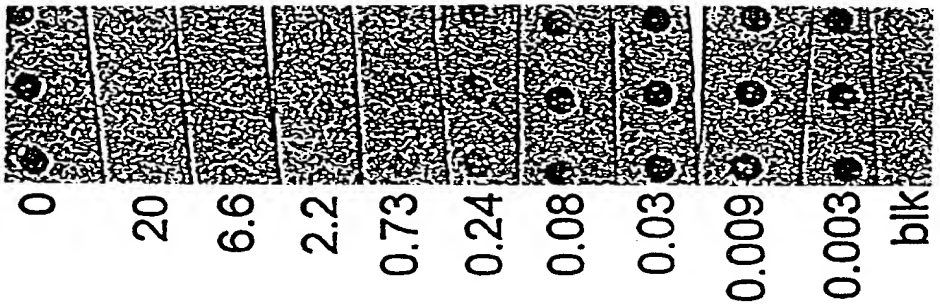

FIG.9

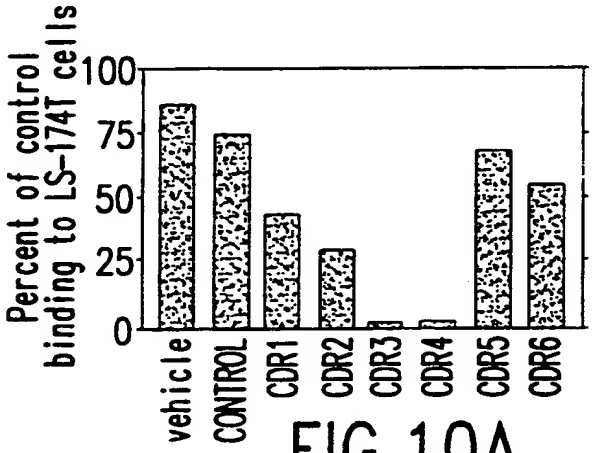

FIG.10A

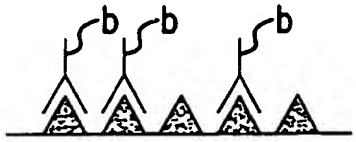

FIG.10C

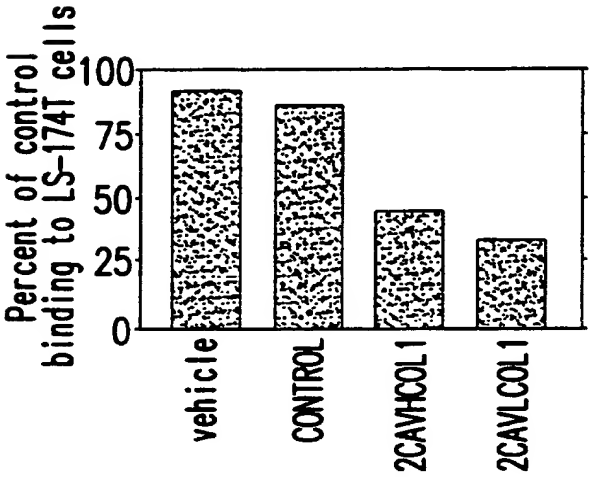

FIG.10B

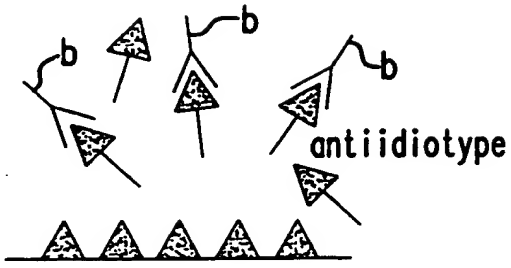

FIG.10D

cstacy
DSABL-1 GACATTGTGATGTCACAGTCTCCATCCTCCCTAGCTGTGTCAGTTGGAGAGAAGGTTACTATG 0.05
PAGE 63
DSABL-1c
GCAGCTCATAGTAACCTTCTCTCCAACTGACACAGCTAGGGAGGATGGAGACTGTGACATCACAATGTCTGC
TTGGGC 0.05 PAGE 78
MSAL-CDR1-1 GCT in MSAL VAC-CDR1-1
AGCTGCGTCGGCAGCCTCCGAAGCAGCCCGCTCCAGAGCCCGCTGCTCCGATGGTACCAGCAGAAACCAG
GGCAGTCTCCTAAA 0.05 PAGE 84
MSAL-CDR1-1c
CTGCCCTGGTTTCTGCTGGTACCATCGGAGCAGCGGGCTCTGGAGCGGGCTCCTTCGGAGGCTGCCGAC
0.05 PAGE 69

HMVL1 GACATTGTGATGTCACAGTCTCCATCGTCCCTAGCTGTGTCAGTTGGAGAGAAGGTTACTATGAGCGCTAAGTCCAGT
HMVL2 CAGAGCCTTTTATATAGTAGCAATCAAAAGATCTACTTGGCCTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAA
HMVL3 CTGCTGATTTACTGGGCATCCACTAGGGAATCTGGGGTCCCTGATCGCTTCACAGGCGGTGGATCTGGG
HMVL5 GCACAGCAATATTATAGATATCCTCGGACGTTCGGTGGAGGCACCAAGCTGGAAATCAAACGGGAATTC
HMVL8 ACCGCCTGTGAAGCGATCAGGGACCCCAGATTCCCTAGTGGATGCCCAGTAAATCAGGAGTTTAGGAGA
HMVL9 CTGCCCTGGTTTCTGGTGGTACCAGGCCAAGTAGATCTTTTGATTGCTACTATATAAAAGGCTCTGACTGGACTT
HMVL10 AGCGCTCATAGTAACCTTGTCTCCAACTGACACAGCTAGGGAGGATGGAGACTGTGACATCACAATGTCTGCTTGGGC
HMVL6 GAATTCCCGTTTGATTTCCAGCTTGGTGCCTCCACCGAACGTCCGAGGATATCTATAATATTGCTGTGGGTAATAAAC

HMVL4
AG AGA TTT CAG TCT CAC CAT CAG CAG TGT GAA GGC TGA AGA CCT GGC
AGT TTA TTA C

HMVL7
TG CCA GGT CTT CAG CCT TCA CAC TGC TGA TGG TGA GAG TGA AAT CTG
TCC CAG ATC C

FIG.11

MSA-63 epitope DNA
GTC GGC AGC CTC CGA AGC AGC CCG CTC CAG AGC CCG CTG CTC CGA CCG CTC GTC
CAG AGC AGC CTC TGC TTG CTG TTC CTC TTG CTG CGA TAC AGC TGC GGC GAC GGC AGC
TGC AGC CGA CGA TAC TGC GAC TTG ACG GTG TGC ACG CGA ATG TAC TTG CTG CTG
CGA TTC ACG GAC GCG CCG CTC CCG CAG ACG TGC TGC GTC TTG AGC

FIG.12A

MSA-63 protein sequence(Start residue 143 end residue 233)
Gln Pro Ser Glu Ala Ser Ser Gly Glu Val Ser Gly Asp Glu Ala Gly Glu Gln Val Ser Ser Glu Thr Asn Asp
Lys Glu Asn Asp Ala Met Ser Thr Pro Leu Pro Ser Thr Ser Ala Ala Ile Thr Leu Asn Cys His Thr Cys Ala
Tyr Met Asn Asp Asp Ala Lys Cys Leu Arg Gly Glu Gly Val Cys Thr Thr Gln Asn Ser

FIG.12B

MSA-63 oligo

MSA1
GTC GGC AGC CTC CGA AGC AGC CCG CTC CAG AGC CCG CTG CTC CGA

MSA2
AGC CCG CTG CTC CGA CCG CTC GTC CAG AGC AGC CTC TGC TTG CTG

MSA3
AGC CTC TGC TTG CTG TTC CTC TTG CTG CGA TAC AGC TGC GGC GAC

MSA4
TAC AGC TGC GGC GAC GGC AGC TGC AGC CGA CGA TAC TGC GAC TTG

MSA5
CGA TAC TGC GAC TTG ACG GTG TGC ACG CGA ATG TAC TTG CTG CTG

MSA6
ATG TAC TTG CTG CTG CGA TTC ACG GAC GCG CCG CTC CCG CAG ACG

MSA7
CGA TTC ACG GAC GCG CCG CTC CCG CAG ACG TGC TGC GTC TTG AGC

FIG.12C

SP-10 Epitope
GAA TTC CAG CCT TCA GGT GAA CAT GGC TCC GGT GAA CAG CCT TCT GGT GAG CAG
GCC TCG GGT GAA CAG CCT TCA GGT GAG CAC GCT TCA GGG GAA CAG GCT TCA GGT
GCA CCA ATT TCA AGC ACA TCT ACA GGC ACA ATA TTA AAT TGC TAC ACA TGT GCT TAT
ATG AAT GAT CAA GGA AAA TGT CTT CGT GGA GAG GGA ACC TGC ATC ACT CAG AAT TC

FIG. 13A

SP-10 protein sequence
Gln Pro Ser Gly Glu His Gly Glu Gln Pro Ser Gly Glu Gln Ala Ser Gly Glu Gln Pro Ser gly Glu His Ala
Ser Gly Glu Gln Ala Ser Gly Ala Gln Ile Ser Ser Thr Ser Thr Gly Thr Ile Leu Asn Cys Tyr Thr Cys Ala
Tyr Met Asn Asp Gln Gly Lys Cys Leu Arg Gly Glu Gly Thr Cys Ile Thr Gln Asn

FIG. 13B

Oligo SP1:
GAA TTC CAG CCT TCA GGT GAA CAT GGC TCC GGT GAA CAG CCT TCT GGT GAG CAG
GCC TCG GGT GAA CAG CCT TAG

Oligo SP2:
GTG AGC ACG CTT CAG GGG AAC AGG CTT CAG GTG CAC CAA TTT CAA GCA CAT CTA
CAG GCA CAA TAT TAA ATT GCT

Oligo SP3:
ACA CAT GTG CTT ATA TGA ATG ATC AAG GAA AAT GTC TTC GTG GAG AGG GAA CCT
GCA TCA CTC AGA ATT C

Oligo SP3a(3Cys->Ala):
ACA CAG CAG CTT ATA TGA ATG ATC AAG GAA AAG CAC TTC GTG GAG AGG GAA
CCG CAA TCA CTC AGA ATT C

Oligo SP4:
GAA TTC TGA GTG ATG CAG GTT CCC TCT CCA CGA AGA CAT TTT CCT TGA TCA TTC ATA
TAA GCA CAT GTG TAG CAA TTT A

Oligo SP4a(3Cys->Ala):
GAA TTC TGA GTG ATT GCC GTT CCC TCT CCA CGA AGT GCT TTT CCT TGA TCA TTC ATA
TAA GCT GCT GTG TAG CAA TTT A

Oligo SP5:
ATA TTG TGC CTG TAG ATG TGC TTG AAA TTG GTG CAC CTG AAG CCT GTT CCC CTG AAG
CGT GCT CAC CTG AAG GCT

Oligo SP6:
GTT CTC CCG AGG CCT GCT CAC CAG AAG GCT GTT CAC CGG AGC CAT GTT CAC CTG
AAG GCT GGA ATT C

FIG. 13C

LHD-C_4Epitope

Oligo LDH1:
TCG TGC CAG TTC CTC GTC GAC TAG CTC TTC GAC TAG CTC CTG CTG CTC TTG TCG GTC ACG GAA TTC

Oligo LDH2:
GAA TTC CGT GAC CGA CAA GAG CAG CAG GAG CTA GTC GAA GAG CTA GTC GAC GAG GAA CTG GCA CGA CGG GTT CGT

FIG.14

Leader:

-19 -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA

V_L:

1 10 20
Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala Val Ser Val Gly Glu Lys Val Thr
GAC ATT GTG ATG TCA CAG TCT CCA TCC TCC CTA GCT GTG TCA GTT GGA GAG AAG GTT ACT

21 27 A B C D E F 30 40
Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser Ser Asn Gln Lys Ile Tyr Leu Ala Trp Tyr Gln Gln Lys Pro
ATG AGC TGC AAG TCC AGT CAG AGC CTT TTA TAT AGT AGC AAT CAA AAG ATC TAC TTG GCC TGG TAC CAG CAG AAA CCA
CDR1
41 50 60
Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Asp
GGG CAG TCT CCT AAA CTG CTG ATT TAC TGG GCA TCC ACT AGG GAA TCT GGG GTC CCT GAT
CDR2
61 70 80
Arg Phe Thr Gly Gly Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Lys Ala
CGC TTC ACA GGC GGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGT GTG AAG GCT

81 90 100
Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr Arg Tyr Pro Arg Thr Phe Gly Gly
GAA GAC CTG GCA GTT TAT TAC TGT CAG CAA TAT TAT AGA TAT CCT CGG ACG TTC GGT GGA
CDR3
101
Gly Thr Lys Leu Glu Ile Lys Arg
GGC ACC AAG CTG GAA ATC AAA CGG

Ala
GCT
invaccine

Met Ser Cys Lys...
ATG AGC TGC AAG...

FIG.15

2CAVHCOL1

VHC1 5'GAATTCATGGCTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGCCCAAAGTGCCC
AAGCACAGATCCAGTTGGTGCA3'

VHC2 5'GTCTGGACCTGAGCTGAAGAAGCCTGGAGAGACAGTCAAGATCTCCGCTAAGGCTTC
TGGGTATACCTTCACAAACTAG3'

VHC3 5'GAATGAACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGGGCTGGAT
AAACACCTACACTGGAGAGCCAACA3'

VHC4 5'TATGCTGATGACTTCAAGGGACGGTTTGCCTTCTCTTTGGAAACCTCTGCCAGCACT
GCCTATTTGCAGATCAACACCT3'

VHC5 5'CAAAAATGAGGACACGGCTACATATTTCGCTGCAAGAGCCTACTATGGTAAATAC
TTTGACTACGAATTC3'

VHC6 5'GAATTCGTAGTCAAAGTATTTACCATAGTAGGCTCTTGCAGCAAATATG3'

VHC7 5'TAGCCGTGTCCTCATTTTTGAGGTTGTTGATCTGCAAATAGGCAGTGCTGGCAGA
GGTTTCCAAAGAGAAGGCAAACCGT3'

VHC8 5'CCCTTGAAGTCATCAGCATATGTTGGCTCTCCAGTGTAGGTGTTTATCCAGCCCAT
CCACTTTAAACCCTTTCCTGGAGC3'

VHC9 5'CTGCTTCACCCAGTTCATTCCATAGTTTGTGAAGGTATACCCAGAAGCCTTAGCGG
AGATCTTGACTGTCTCTCCAGGCT3'

VHC10 5'TCTTCAGCTCAGGTCCAGACTGCACCAACTGGATCTGTGCTTGGGCACTTTGGGC
AGCTGCCATCAGGAATAGCAAGGTCCACACCCAAGCCATGAATTC3'

FIG.16A

2CAVLCOL1

VLC1 5'AGTATTGTGATGACCCAGACTCCCAAATTCCTGCTTGTATCAGCAGGAGACAGGGTT
ACCATA3'

VLC2 5'ACCTGCAAGGCCAGTCAGAGTGTGAGTAATGATGTAGCTTGGTACCAACAGAAAACC
AGGGCAG3'

VLC3 5'TCTCCTAAACTGCTGATATACTATGCATCCAATCGCTACACTGGAGTCCCTGATCGCT
TCACTGGCAGT3'

VLC4 5'GGATATGGGACGGATTTCACTTTCACCATCAGCACTGTGCAGGCTGAAGACCTGGCA
GTTTAT3'

VLC5 5'TTCTGYCAGCAGGATTATAGCTCTCCGCTCACGTTCGGTGCTGGGACCAAGCTGGAG
CTGAAAGAATTC3'

VLC6 5'GAATTCTTTCAGCTCCAGCTTGGTCCCAGCACCGAACGTGAGCGGAGAGCTATAATC
CTGCTGACAGAAATAAACTGC3'

VLC7 5'CAGGTCTTCAGCCTGCACAGTGCTGATGGTGAAAGTGAAATCCGTCCCATATCCA
CTGCCAGT3'

VLC8 5'GAAGCGATCAGGGACTCCAGTGTAGCGATTGGATGCATAGTATATCAGCAGTTTAG
GAGACTGCCCTGG3'

VLC9 5'TTTCTGTTGGTACCAAGCTACATCATTACTCACACTCTGACTGGCCTTGCAGGTTA
TGGTAAC3'

VLC10 5'CCTGTCTCCTGCTGATACAAGCAGGAATTTGGGAGTCTGGGTCATCACAATACTT
GCTTGGGC3'

VLC11 5'TTCGCTCAGCAGGATTATAGCTCTCCGCTCACGTTCGGTGCTGGGACCAAGCTGG
AGCTGAAAGAATC3'

VLC12 5'GAATTCTTTCAGCTCCAGCTTGGTCCCAGCACCGAACGTGAGCGGAGAGCTATAA
TCCTGCTGAGCGAAATAAACTGC3'

FIG.16B

INTERNATIONAL SEARCH REPORT

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/131.1, 133.1, 134.1; 530/350, 387.1, 387.2, 388.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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Medline, West

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	US 5,637,300 (DUNBAR et al.) 10 June 1997 (10.06.1997), column 10-11	1,4,5,10,21,22,25,26, 31 ——— 11,14,15,20
Y	SEFERIAN et al. Antibody synthesis induced by endogenous internal immages. Applied Biochemisrty and Biotechnology 1994, Vol. 47, see pages 213-227.	1, 7-11, 17-21, 28-31
Y	CARRON et al. Characterization of antibodies to idiotypic determinants of monoclonal anti-sperm antibodies. Biology of Reproduction 1988, Vol. 38, see abstract.	1,2,5,10-12,15, 20-23, 26,31
Y	TRIPATHI et al. Antigen mimicry by an anti-idiotypic antibody single chain variable fragment. Molecular Immunology 1998, Vol. 35, see pages 853-863.	1,7,8-11,17-22,28-31
Y	US 5,208,146A (IRIE) 04 May 1993 (04.05.1993) see column 1 and 5.	1,7-11, 17-21, 28-31
Y	US 5,436,157A (HERR et al.) 25 July 1995 (25.07.1995) see columns 1-3.	2,3,6,12,13,16,23,24, 27

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(54) Title: CONTRACEPTIVE ANTIBODY VACCINES

(57) **Abstract**

The invention provides an antibody contraceptive vaccine comprising an antibody that has at least one CDR containing a portion of an antigen of a cell or protein associated with reproductive function and which antibody has an enhanced ability to elicit an anti–idiotype response, for example, by substituting one or more variable region cysteine residues that form intrachain disulfide bonds with an amino acid residue that does not have a sulfhydryl group, such that the intrachain disulfide bond does not form. The invention further provides methods of contraception using the antibody contraceptive vaccines of the invention.

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CONTRACEPTIVE ANTIBODY VACCINES

1. FIELD OF THE INVENTION

The present invention relates to modified antibodies, and vaccine compositions thereof, that have one or more complementary determining regions that contain portions of sperm antigens, in which modified antibodies one or more variable region cysteine residues that form intrachain disulfide bonds have been replaced with amino acid residues that do not contain a sulfhydryl group and, therefore, do not form disulfide bonds. The present invention also relates to use of the vaccine compositions of the invention as a contraceptive.

2. BACKGROUND OF THE INVENTION

2.1. IMMUNOGLOBULIN STRUCTURE

The basic unit of immunoglobulin structure is a complex of four polypeptides -- two identical low molecular weight or "light" chains and two identical high molecular weight or "heavy" chains, linked together by both noncovalent associations and by disulfide bonds. Each light and heavy chain of an antibody has a variable region at its amino terminus and a constant domain at its carboxyl terminus (Figure 1). The variable regions are distinct for each antibody and contain the antibody antigen binding site. Each variable domain is comprised of four relatively conserved framework regions and three regions of sequence hypervariability termed complementarity determining regions or CDRs (Figure 2). For the most part, it is the CDRs that form the antigen binding site and confer antigen specificity. The constant regions are more highly conserved than the variable domains, with slight variations due to haplotypic differences.

Based on their amino acid sequences, light chains are classified as either kappa or lambda. The constant region heavy chains are composed of multiple domains (CH1, CH2, CH3...CHx), the number depending upon the particular antibody class. The CH1 region is separated from the CH2 region by a hinge region which allows flexibility in the antibody. The variable region of each light chain aligns with the variable region of each heavy chain, and the constant region of each light chain aligns with the first constant region of each heavy chain. The CH2-CHx domains of the constant region of a heavy chain form an "Fc region" which is responsible for the effector functions of the immunoglobulin molecule, such as complement binding and binding to the Fc receptors expressed by lymphocytes, granulocytes, monocyte lineage cells, killer cells, mast cells and other immune effector cells.

As seen in Figure 3. the light and heavy chains of an IgG molecule form the variable region domain and the constant region domain. Each domain is composed of a sandwich of two parallel extended protein layers of about 100 amino acids in length which are connected by a single disulfide bond (See Roitt et al., Immunology, 3rd Edition, London: Mosby, 1993, p4.4 (Figure 3)). Each of the two extended protein layers of the domain, in turn, contains two "anti-parallel" adjacent strands which adopt a beta-sheet conformation. (See, *e.g.*, Stryer, 1975, Biochemistry, WH Freeman and Co., p. 950). Each of the domains has a similar three-dimensional structure based on the immunoglobulin fold.

2.2. IMMUNOTHERAPY AND ANTI-IDIOTYPE ANTIBODIES

In modern medicine, immunotherapy or vaccination has virtually eradicated diseases such as polio, tetanus, tuberculosis, chicken pox, measles, hepatitis, etc. The approach using vaccinations has exploited the ability of the immune system to prevent infectious diseases.

Use of immunotherapy has also been explored for cancer therapy. The era of tumor immunology began with experiments by Prehn and Main, who showed that antigens on the methylcholanthrene (MCA)-induced sarcomas were tumor specific in that transplantation assays could not detect these antigens in normal tissue of the mice (Prehn et al., 1957, J. Natl. Cancer Inst. 18:79-778). This notion was confirmed by further experiments demonstrating that tumor specific resistance against MCA-induced tumors could be elicited in the autochthonous host, that is, the mouse in which the tumor originated (Klein et al., 1990, Cancer Res. 20:151-1572).

There are many reasons why immunotherapy is desired for use in cancer patients. First, if cancer patients are immunosuppressed in surgery, with anesthesia and subsequent chemotherapy, it may worsen the immunosuppression, then with appropriate immunotherapy in the preoperative period, this immunosuppression may be prevented or reversed. This could lead to fewer infectious complications and accelerated wound healing. Second, tumor bulk is minimal following surgery and immunotherapy is most likely to be effective in this situation. A third reason is the possibility that tumor cells are shed into the circulation at surgery and effective immunotherapy applied at this time can eliminate these cells.

There are two types of immunotherapy, the "active immunotherapy" and the "passive immunotherapy". In "active immunotherapy", an antigen is administered in the form of a vaccine, to a patient, so as to elicit a protective immune response. "Passive immunotherapy" involves the administration of antibodies to a patient without eliciting a concommitant immune response. When a specific antibody from one animal is injected as

an immunogen into a suitable second animal, the injected antibody will elicit an immune response. Antibody therapy is conventionally characterized as passive since the patient is not the source of the antibodies. However, the term passive is misleading because the patient can produce anti-idiotypic secondary antibodies which in turn provoke an immune response which is cross-reactive with the original antigen. Immunotherapy where the patient generates secondary antibodies is often more therapeutically effective than passive immunotherapy because the patient's own immune system continues to fight the cells bearing the particular antigen well after the initial infusion of antibody.

In an anti-idiotype response, antibodies produced initially during an immune response or introduced into an organism will carry unique new epitopes to which the organism is not tolerant, and therefore will elicit production of secondary antibodies (termed "Ab2"), some of which are directed against the idiotype (*i.e.*, the antigen binding site) of the primary antibody (termed "Ab1"), *i.e.*, the antibody that was initially produced or introduced exogenously. These secondary antibodies or Ab2 likewise will have an idiotype, which will induce production of tertiary antibodies (termed "Ab3"), some of which will recognize the antigen binding site of Ab2, and so forth. This is known as the "network" theory. Some of the secondary antibodies will have a binding site which is an analog of the original antigen, and thus will reproduce the "internal image" of the original antigen. And, the tertiary or Ab3 antibodies that recognize this antigen binding site of the Ab2 antibody will also recognize the original antigen (Figure 4).

Therefore, anti-idiotypic antibodies have binding sites that are similar in conformation and charge to the antigen, and can elicit the same or greater response than that of the cancer antigen itself. Administration of an exogenous antibody that can elicit a strong anti-idiotypic response can thus serve as an effective vaccine, by maintaining a constant immune response.

To date, anti-idiotypic vaccines have comprised murine antibodies because the anti-idiotypic response occurs as part of the typical human anti-mouse antibody (HAMA) response. A strong anti-idiotypic cascade has been observed when Ab1 has been structurally damaged (Madiyalakan et al., 1995, *Hybridoma* 14:199-203), rendering the antibody more foreign. There has been direct administration to the subject of exogenously produced anti-idiotype antibodies that are raised against the idiotype of an anti-tumor antibody (U.S. Patent No. 4,918,14). After administration, the subject's body will produce anti-antibodies which not only recognize these anti-idiotype antibodies, but also recognize the original tumor epitope, thereby directing complement activation and other immune system responses to a foreign entity to attack the tumor cell that expresses the tumor epitope.

However, while anti-idiotypic vaccines are desirable targets and several have been identified, the ability to deliver antibodies that reproducibly cause the generation of such an anti-idiotypic response is not currently possible. (Foon et al., 1995, *J. Clin. Invest.* 9:334-342; Madiyalakan et al., 1995, *Hybridoma* 14:199-203). One of the reasons for the failure to generate an anti-idiotypic response is that, Ab1, while exogenous, is still very similar to "self", as all antibodies have very similar structures, and anti-idiotypic responses to self molecules tend to be very limited. Thus, there is a need in the art for methods of reliably generating an anti-idiotype response to a specific antibody.

2.3. CONTRACEPTIVE METHODS

A variety of contraceptive methods are currently available. Such methods include barrier methods such as condoms or diaphragms, or use of spermicidal agents such as non-oxynol-9, hormone therapies such as birth control pills or implants, and other methods such as intrauterine devices. All of these methods pose problems as convenient and effective methods of preventing conception. Some methods are inconvenient or ineffective, some pose health risks, while others are costly. Accordingly, there is a need in the art for a safe, inexpensive, and convenient method of contraception.

3. SUMMARY OF THE INVENTION

The present invention is based upon the realization of the present inventors that an antibody in which one or more variable region cysteine residues that form one or more intrachain disulfide bonds have been replaced with amino acid residues that do not contain sulfhydryl groups, such that the particular disulfide bonds do not form, elicit a much stronger anti-idiotype response than an antibody in which the variable region disulfide bonds are intact. Additionally, the present inventors have realized that portions of antigens of proteins or reproductive cells, particularly sperm antigens, can be inserted into or used to replace portions of one or more complementarity determining regions, such that the modified antibody can be used as a vaccine to generate anti-idiotype antibodies that recognize the particular antigen.

Accordingly, the present invention provides modified immunoglobulin molecules or antibodies (and functionally active fragments, derivatives and analogs thereof), and vaccine compositions containing these immunoglobulin molecules, wherein the variable region of the immunoglobulin is subject to decreased conformational constraints, such as, but not limited to, by breaking one or more intrachain or interchain disulfide bonds. Specifically, the invention provides modified immunoglobulins that comprise a variable region and are identical, except for one or more amino acid substitutions in said variable region, to a

second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding (*i.e.*, specific binding of the immunoglobulin to its antigen as determined by any method known in the art for determining antibody-antigen binding, which excludes non-specific binding but not necessarily cross-reactivity with other antigens) an antigen or having a CDR that contains a portion of an antigen, said one or more amino acid substitutions being the substitution of one or more amino acid residues that do not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule. In preferred embodiments, the second immunoglobulin molecule contains a CDR that contains a portion of an antigen of a cell or protein involved in reproductive function, preferably sperm antigens, more preferably the sperm antigens SP-10, LDH-C_4, or MSA-63.

The invention further provides methods of eliciting an anti-idiotype response in a subject by administering the modified immunoglobulins of the invention. In particular, the modified immunoglobulins of the invention can be used as contraceptives, either in males or, preferably in females, specifically by administering an immunoglobulin molecule of the invention, which immunoglobulin molecule was derived (*i.e.*, by modification according to the invention to replace one or more variable region cysteine residues that form an intrachain disulfide bond with an amino acid residue that does not contain a sulfhydryl group) from an immunoglobulin molecule that contains a CDR that contains a portion of an antigen of a protein or cell associated with reproductive function, preferably a sperm antigen.

The invention also provides methods of producing the modified immunoglobulin molecules of the invention and vaccine compositions containing the modified immunoglobulin molecules of the invention.

4. DESCRIPTION OF FIGURES

Figure 1. A schematic diagram showing the structure of the light and heavy chain of an immunoglobulin molecule, each chain consisting of a variable region positioned at the amino terminal region (H_2N-) and a constant region positioned at a carboxyl terminal region (-COOH).

Figure 2. A schematic diagram of an IgG showing the four framework regions (FR1, FR2, FR3 and FR4) and three complementarity determining regions (CDR1, CDR2 and CDR3) in the variable regions of the light and heavy chains (labeled as V_L and V_H, respectively). The constant region domains are indicated as C_L for the light chain constant domain and CH_1, CH_2 and CH_3 for the three domains of the heavy chain constant region. Fab indicates the portion of the antibody fragment which includes the variable region

domains of both light and heavy chains and the C_L and CH_1 domains. Fc indicates the constant region fragment containing the CH_2 and CH_3 domains.

Figure 3. A schematic diagram of an antibody structure as shown in Figure 2, but drawn to emphasize that each domain (the loop structures labeled as V_L, V_H, C_L, CH_1, CH_2, and CH_3, respectively) is structurally defined by a disulfide bond (indicated with darkest lines) that maintains the three-dimensional structure (Roitt et al., *Immunology*, Second Edition, London: Gower Medical Publishing, 1989, p 5.3).

Figure 4. A schematic diagram showing the development of internal image bearing anti-idiotype antibodies (Ab2) and anti-anti-idiotype antibodies (Ab3) from idiotype antibodies (Ab1) directed against an antigen of a tumor cell in an antiidiotypic cascade.

Figure 5. Modification of the variable region of an immunoglobulin by replacing cysteine residues in the variable regions with alanine residues to remove an intrachain disulfide bond. CH1, CH2 and CH3 are constant regions. V_H is the heavy chain variable region and V_L is the light chain variable region.

Figures 6A-C. (A). The structure of the expression vector pMRRO10.1, which contains a human kappa light chain constant region sequence. (B). The structure of the expression vector pGamma1 that contains a sequence encoding a human IgG1 constant region (CH1, CH2, CH3) heavy chain and hinge region sequences. (C) The structure of the expression vector pNEPuDGV which contains a sequence encoding the kappa constant domain of the light chain and the constant domain and hinge region of the heavy chain. For all three vectors see Bebbington et al., 1991, *Methods in Enzymology* 2:136-145.

Figures 7A and B. (A) The amino acid sequence and corresponding nucleotide sequence for the consensus light chain variable region ConVL1. (B) The amino acid and corresponding nucleotide sequences for the consensus heavy chain variable region ConVH1.

Figure 8. A schematic diagram of the general steps that were followed for the assembly of an engineered gene encoding the synthetic modified antibody specific to human colon cancer antigen.

Figure 9. Dot blot showing the result of an assay for the competition of binding of the antibody derived from mAB31.1, but not having the cysteine to alanine changes with the same antibody which is biotin labeled to an antigen preparation derived from LS-174 T-cells. The concentration of the unlabeled antibody is indicated as nM unlabeled antibody. The "blk" lane has no antigen.

Figures 10A-D. (A)Results of competition binding assay of the biotin-labeled anti-colon carcinoma cell antibody to LS-174T cells in the presence of antisera from mice vaccinated with vehicle alone, control antibody that binds the colon carcinoma cell antibody

but has not been modified, and peptides CDR1, CDR2, CDR3, CDR4, CDR5, and CDR6, having the CDR sequences containing the bradykinin receptor binding site expressed as percent of control binding to LS-174T cells. (B). Results of competition binding assays of the biotin-labeled anti-colon carcinoma cell antibody to LS-174T cells in the presence of antisera from mice vaccinated with vehicle alone, control antibody that binds the colon carcinoma cell antibody, but has not been modified, 2CAVHCOL1, and 2CAVLCOL1. (C) Diagram showing the binding of a biotin-labeled (indicated by the "b") antibody (inverted Y) to antigen (solid triangles). (D) Diagram showing the inhibition of binding of the biotin-labeled (indicated by the "b") antibody (inverted Y) by anti-idiotype antibodies (solid arrows) to antigen (solid triangles).

Figure 11. Nucleotide sequences of the oligonucleotides used to construct the MSA1 and MSALVAC-1 variable regions.

Figures 12A-C. (A) Nucleotide sequence for the MSA-63 epitope. (B) Amino acid sequence of the MSA-63 epitope encoded by the nucleotide sequence of Figure 12A. (C) MSA-63 oligonucleotides used to construct a modified variable region. Each oligo overlaps for five codons and transitions the entire sequence of Figure 12A.

Figures 13 A-C. (A) Nucleotide sequence for the SP-10 epitope. (B) Amino acid sequence of the SP-10 epitope encoded by the nucleotide sequence of Figure 13A. (C) Oligonucleotides of Sp-10 used to construct a modified variable region. SP3a and SP4a are modified to change the codons encoding certain cysteine residues to codons encoding alanine residues.

Figure 14. Oligonucleotides of LDH-C4 epitope sequence for construction of a modified variable region gene containing a LDH-C_4.

Figure 15. Nucleotide and amino acid sequence of the consensus contraceptive light chain variable region.

Figure 16 A-B. (A) Sequences of oligos used in the construction of 2CAVHCOL1. (B) Sequences of oligos used in the construction of 2CAVLCOL1.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention provides modified immunoglobulins (particularly antibodies and functionally active fragments, derivatives, and analogs thereof) that can be used as contraceptive vaccines. Specifically, these antibodies have one or more complementarity determining regions (CDRs) that contain a portion of an antigen of a cell or protein involved in reproductive functio, preferably a sperm antigen. In addition, these antibodies have been engineered to elicit a stronger immune response, particularly a stronger anti-idiotypic response, than the corresponding unmodified immunoglobulins. In particular, the

modified immunoglobulins of the invention are immunoglobulins that are modified to decrease the conformational constraints on one variable region of the immunoglobulin molecule, preferably, such that at least one of the cysteines that participates in forming an intrachain disulfide bond in the variable region of the immunoglobulin has been replaced with an amino acid residue that does not have a sulfhydryl group and, therefore, does not form a disulfide bond, thereby decreasing the conformational constraints of at least one of the variable regions of the immunoglobulin (Figure 5).

The invention also provides vaccine compositions containing the modified immunoglobulin molecules of the invention. Additionally, the invention provides methods of generating an anti-idiotype response in a subject by administration of the modified immunoglobulin molecules of the invention.

In specific embodiments, the invention provides methods of contraception by administration of a modified immunoglobulin molecule of the invention which, in its unmodified state, is capable of immunospecifically binding an antigen of a protein or cell associated with reproductive function, such as a sperm antigen. Administration of the modified immunoglobulin elicits an anti-idiotype reaction in the subject, leading to the production, by the subject, of antibodies specific for the particular antigen.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

5.1. MODIFIED ANTIBODIES

The modified immunoglobulins, particularly antibodies, of the invention are immunoglobulins that, at least in the unmodified state, can immunospecifically bind an antigen of a cell or protein associated with reproductive function, and have been modified to enhance their ability to elicit an anti-idiotype response. Such immunoglobulins are modified to reduce the conformational constraints on a variable region of the immunoglobulin, *e.g.*, by removing or reducing intrachain or interchain disulfide bonds. Specifically, the invention provides a first immunoglobulin molecule that comprises a variable region and that is identical, except for one or more amino acid substitutions in the variable region, to a second immunoglobulin molecule, the second immunoglobulin molecule being capable of immunospecifically binding an antigen, the amino acid substitutions being the substitution of one or more amino acid residues that do not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule. (See, co-pending United States Patent Application Serial No., entitled "Modified Antibodies With Enhanced Ability To Elicit An Anti-Idiotype Response", filed November 13, 1998 (attorney docket

no. 6750-015), which is incorporated by reference herein in its entirety. The invention also provides nucleic acids containing a nucleotide sequence encoding a modified immunoglobulin of the invention.

Identifying the cysteine residues that form a disulfide bond in a variable region of a particular antibody can be accomplished by any method known in the art. For example, but not by way of limitation, it is well known in the art that the cysteine residues that form intrachain disulfide bonds are highly conserved among antibody classes and across species. Thus, the cysteine residues that participate in disulfide bond formation can be identified by sequence comparison with other antibody molecules in which it is known which residues form a disulfide bond.

Table 1 provides a list of the positions of disulfide bond forming cysteine residues for a number of antibody molecules.

Table 1 (derived from Kabat et al, 1991, sequences of Proteins of Immunological Interest, 5th Ed., U.S. Department of Health and Human Services, Bethesda, Maryland).

Species	Variable domain	Subgroup	Disulfide bond-forming cysteines (positions)
Human	kappa light	I	23,88
Human	kappa light	II	23,88
Human	kappa light	III	23,88
Human	kappa light	IV	23,88
Human	lambda light	I	23,88
Human	lambda light	II	23,88
Human	lambda light	IIII	23,88
Human	lambda light	IV	23,88
Human	lambda light	V	23,88
Human	lambda light	VI	23,88
Mouse	kappa light	I	23,88
Mouse	kappa light	II	23,88
Mouse	kappa light	III	23,88
Mouse	kappa light	IV	23,88
Mouse	kappa light	V	23,88
Mouse	kappa light	VI	23,88
Mouse	kappa light	VII	23,88
Mouse	kappa light	Miscellaneous	23,88
Mouse	lambda light		23,88
Chimpanzee	lambda light		23,88
Rat	kappa light		23,88
Rat	lambda light		23,88
Rabbit	kappa light		23,88
Rabbit	lambda light		23,88

Species	Variable domain	Subgroup	Disulfide bond-forming cysteines (positions)
Dog	kappa light		23,88
Pig	kappa light		23 (88)
Pig	lambda light		23,88
Guinea pig	lambda light		23 (88)
Sheep	lambda light		23,88
Chicken	lambda light		23,88
Turkey	lambda light		23 (88)
Ratfish	lambda light		23 (88)
Shark	kappa light		23,88
Human	heavy	I	22,92
Human	heavy	II	22,92
Human	heavy	III	22,92
Mouse	heavy	I (A)	22,92
Mouse	heavy	I (B)	22,92
Mouse	heavy	II (A)	22,92
Mouse	heavy	II (B)	22,92
Mouse	heavy	II (C)	22,92
Mouse	heavy	III (A)	22,92
Mouse	heavy	III(B)	22,92
Mouse	heavy	III (C)	22,92
Mouse	heavy	III (D)	22,92
Mouse	heavy	V (A)	22,92
Mouse	heavy	V (B)	22,92
Mouse	heavy	Miscellaneous	22,92
Rat	heavy		22,92
Rabbit	heavy		22,92
Guinea pig	heavy		22,92
Cat	heavy		22 (92)
Dog	heavy		22,92
Pig	heavy		22 (92)
Mink	heavy		22 (92)
Sea lion	heavy		22 (92)
Seal	heavy		22 (92)
Chicken	heavy		22,92
Duck	heavy		22 (92)
Goose	heavy		22 (92)
Pigeon	heavy		22 (92)
Turkey	heavy		22 (92)
Caiman	heavy		22, 92
Xenopus frog	heavy		22,92
Elops	heavy		22,92
Goldfish	heavy		22,92
Ratfish	heavy		22 (92)
Shark	heavy		22,92

Position numbers enclosed by () indicate that the protein was not sequenced to that position, but the residue is inferred by comparison to known sequences.

Notably, for all of the antibody molecules listed in Table 1, the cysteine residues that form the intrachain disulfide bonds are the residues at positions 23 and 88 of the light chain variable domain and the residues at positions 22 and 92 of the heavy chain variable domain. The position numbers refer to the residue corresponding to that residue in the consensus sequences as defined in Kabat, (1991, Sequences of Proteins of Immunological Interest, 5th Ed., U.S. Department of Health and Human Services, Bethesda, Maryland) or as indicated in the heavy and light chain variable region sequences depicted in Figures 7A and B, respectively ("corresponding" means as determined by aligning the particular antibody sequence with the consensus sequence or the heavy or light chain variable region sequence depicted in Figure 7A or B).

Accordingly, in one embodiment of the invention, the modified immunoglobulin molecule is an antibody in which the residues at positions 23 and/or 88 of the light chain are substituted with an amino acid residue that does not contain a sulfhydryl group and/or the residues at positions 22 and/or 92 of the heavy chain are substituted with an amino acid residue that does not contain a sulfhydryl group.

In the modified immunoglobulin of the invention, the amino acid residue that substitutes for the disulfide bond forming cysteine residue is any amino acid residue that does not contain a sulfhydryl group, *e.g.*, alanine, arginine, asparagine, aspartate (or aspartic acid), glutamine, glutamate (or glutamic acid), glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine or valine. In a preferred embodiment, the cysteine residue is replaced with a glycine, serine, threonine, tyrosine, asparagine, or glutamine residue, most preferably, with an alanine residue.

Additionally, the disulfide bond forming cysteine residue may be replaced by a nonclassical amino acid or chemical amino acid analog that does not contain a sulfhydryl group (for example, but not by way of limitation, using routine protein synthesis methods). Non-classical amino acids include, but are not limited, to the D-isomers of the common amino acids, α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-aminobutyric acid, γ-Abu, ϵ-Ahx, -amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary). In an alternative embodiment, the disulfide bond forming residue is deleted.

In specific embodiments, the substitution of the disulfide bond forming residue is in the heavy chain variable region or is in the light chain variable region or is in both the heavy chain and light chain variable regions. In other specific embodiments, one of the residues that forms a particular disulfide bond is replaced (or deleted) or, alternatively, both residues that form a particular disulfide bond may be replaced (or deleted).

In other embodiments, the invention provides immunoglobulin molecules that have one or more amino acid substitutions relative to the second immunoglobulin molecule of a disulfide bond forming residue in the variable region with an amino acid residue that does not contain a sulfhydryl group and that additionally have one or more other amino acid substitutions (*i.e.*, that are not a replacement of a disulfide bond forming residue with a residue that does not contain a sulfhydryl group).

In particular, the invention provides a first immunoglobulin molecule comprising a variable region and which is identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding an antigen of a cell or protein associated with reproductive function or that has at least one CDR that contains a portionof an antigen of a cell or protein associated with reproductive function, in which at least one of said one or more amino acid substitutions are the substitution of an amino acid residue that does not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule.

In a preferred embodiment, the amino acid substitutions that are not the substitution of a disulfide bond forming cysteine residue with a residue that does not have a sulfhydryl group, are not stabilizing changes. Stabilizing changes are defined as those amino acid changes that increase the stability of the antibody molecule. Such stabilizing amino acid changes are those changes that substitute an amino acid that is not common at that particular position in the particular antibody molecule (*e.g.*, as defined by the consensus sequences for a number of antibody molecules provided in Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., U.S. Department of Health and Human Services, Bethesda, Maryland) with a residue that is common at that particular position, *e.g.*, is the amino acid at that position in the consensus sequence for that antibody molecule (see PCT Publication WO 96/02574, dated February 1, 1996 by Steipe et al.).

Such other amino acid substitutions can be any amino acid substitution that does not alter the ability of the modified immunoglobulin to elicit the formation of anti-anti-idiotype antibodies, *e.g.*, as determined, for example, as described in Section 5.5, *infra*. For example, such other amino acid substitutions include substitutions of functionally equivalent amino acid residues. For example, one or more amino acid residues can be

substituted by another amino acid of a similar polarity which acts as a functional equivalent. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

The modified immunoglobulin is derived from an antibody that has one or more CDRs containing a portion of an antigen of a cell or protein associated with reproductive function. In specific embodiments, the antigen is a sperm antigen, preferably SP-10. Other antigens include lactate dehydrogenase LDH-C4, SP-17, PH-20, FA-1, FA-2, PH-30, RSA, HAS-63, MSA-63, or zona pellucida proteins ZP1, ZP2, and ZP3 (see, e.g., Freemerman et al., 1993, Molecular Reproduction and Development 34:140-148; Herr et al., 1990, Biol. Reproduction 42:181-193; O'Hern et al., 1995, Biol. Reproduction 52:331-339; Anderson et al., 1986, J. Reprod. Immunol. 10:231-257; Wright et al., 1990, Biology of Reproduction 42:693-701; Lea et al., 1997, Fertility and Sterility 67:355-361; O'Hern et al., Elsevier Science Ltd. 16:1761-1766; Kerr, 1995, Reprod. Fertil. Dev. 7:825-830; Kaul et al., 1996, Reprod. Fertil Dev. 50:127-134; Liu et al., 1990, Molecular Reproduction and Development 25:302-308; Bambra, 1992, Scand. J. Immunol. 11:118-122) or another antigen of a cell or protein associated with reproductive function, for example but not limited to gonadotropin-releasing hormone, any gonadotropin, prostaglandin F2 alpha, oxytocin, and gonadotropin 1 receptors.

The immunoglobulin molecules of the invention can be of any type, class, or subclass of immunoglobulin molecules. In a preferred embodiment, the immunoglobulin molecule is an antibody molecule, more preferably of a type selected from the group consisting of IgG, IgE, IgM, IgD and IgA, most preferably is an IgG molecule. Alternatively, the immunoglobulin molecule is a T cell receptor, a B cell receptor, a cell-surface adhesion molecule such as the co-receptors CD4, CD8, or CD19, or an invariant domain of an MHC molecule.

The modified immunoglobulin can be derived from any naturally occurring antibody, preferably a monoclonal antibody, or can be derived from a synthetic or engineered antibody. Specifically, the modified immunoglobulin molecules are derived from an antibody in which a portion of an antigen of a cell or protein associated with reproductive function is inserted into or replaces all or a portion of one of the CDRs in the variable region, for example as described in co-pending United States Patent application

Serial No., entitled "Immunoglobulin Molecules Having A Synthetic Variable Region And Modified Specificity", by Burch, filed November 13, 1998 (attorney docket no. 6750-016), which is incorporated by reference herein in its entirety.

In particular, the synthetic antibodies are antibodies that in which at least one of the CDRs of the antibody contains an antigen of a cell or protein associated with reproductive function. In one aspect of the invention, the amino acid sequence of the antigen is not found naturally within the CDR. One or more CDRs may also contain a binding site for a cell or protein involved in reproductive function.

The amino acid sequence of the binding site may be identified by any method known in the art. For example, in some instances, the sequence of a member of a binding pair has already been determined to be directly involved in binding the other member of the binding pair. In this case, such a sequence can be used to construct the CDR of a synthetic antibody that specifically recognizes the other member of the binding pair. If the amino acid sequence for the binding site in the one member of the binding pair for the other member of the binding pair is not known, it can be determined by any method known in the art, for example, but not limited to, molecular modeling methods or empirical methods, *e.g.*, by assaying portions (*e.g.*, peptides) of the member for binding to the other member, or by making mutations in the member and determining which mutations prevent binding.

The binding pair can be any two molecules, including proteins, nucleic acids, carbohydrates, or lipids, that interact with each other, although preferably the binding partner from which the binding site is derived is a protein molecule. In preferred embodiments, the modified immunoglobulin contains a binding sequence for a cancer antigen, an infectious disease antigen, a cellular receptor for a pathogen, or a receptor or ligand that participates in a receptor-ligand binding pair.

In specific embodiments, the binding pair is a protein-protein interaction pair which is either homotypic interaction (*i.e.*, is the interaction between two of the same proteins) or a heterotypic interaction (*i.e.*, is the interaction between two different proteins).

The synthetic antibody may be built upon (*i.e.*, the binding site sequences inserted into the CDR of) the sequence of a naturally occurring or previously existing antibody or may be synthesized from known antibody consensus sequences, such as the consensus sequences for the light and heavy chain variable regions in Figures 7A and B, or any other antibody consensus or germline (*i.e.*, unrecombined genomic sequences) sequences (*e.g.*, those antibody consensus and germline sequences described in Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th edition, NIH Publication No. 91-3242, pp 2147-2172).

Each antibody molecule has six CDR sequences, three on the light chain and three on the heavy chain, and five of these CDRs are germline CDRs (*i.e.*, are directly derived from the germline genomic sequence of the animal, without any recombination) and one of the CDRs is a non-germline CDR (*i.e.*, differs in sequence from the germline genomic sequence of the animal and is generated by recombination of the germline sequences). Whether a CDR is a germline or non-germline sequence can be determined by sequencing the CDR and then comparing the sequence with known germline sequences, *e.g.*, as listed in Kabat et al. (1991, Sequences of Proteins of Immunological Interest, 5th edition, NIH Publication No. 91-3242, pp 2147-2172). Significant variation from the known germline sequences indicates that the CDR is a non-germline CDR. Accordingly, the CDR that contains the amino acid sequence of the binding site or antigen is a germline CDR or, alternatively, is a non-germline CDR.

The binding site or antigen sequence can be inserted into any of the CDRs of the antibody, and it is within the skill in the art to insert the binding site into different CDRs of the antibody and then screen the resulting modified antibodies for the ability to bind to the particular member of the binding pair, *e.g.* as discussed in Section, *infra*, or to elicit an immune response against the antigenic site, *e.g.*, as described in Section, *infra*. Thus, one can determine which CDR optimally contains the binding site or antigen. In specific embodiments, a CDR of either the heavy or light chain variable region is modified to contain the amino acid sequence of the binding site or antigen. In another specific embodiment, the modified antibody contains a variable domain in which the first, second or third CDR of the heavy variable region or the first, second or third CDR of the light chain variable region contains the amino acid sequence of the binding site or antigen. In another embodiment of the invention, more than one CDR contains the amino acid sequence of the binding site or antigen or more than one CDR each contains a different binding site for the same molecule or contains a different binding site for a different molecule. In particular, embodiments, two, three, four, five or six CDRs have been engineered to contain a binding site for the first member of the binding pair. In a preferred embodiment, one of the CDRs contains a portion of one sperm antigen and another CDR contains a portion of a second sperm antigen, more particularly, where one sperm antigen in SP-10 and the other sperm antigen is MSA-63 or LHD-C_4.

In specific embodiments of the invention, the binding site or antigen amino acid sequence is either inserted into the CDR without replacing any of the amino acid sequence of the CDR itself or, alternatively, the binding site or antigen amino acid sequence replaces all or a portion of the amino acid sequence of the CDR. In specific embodiments, the

binding site amino acid sequence replaces 1, 2, 5, 8, 10, 15, or 20 amino acids of the CDR sequence.

The amino acid sequence of the binding site or antigen present in the CDR can be the minimal binding site necessary for the binding of the member of the binding pair or for eliciting an immune response against the antigen(which can be determined empirically by any method known in the art); alternatively, the sequence can be greater than the minimal binding site or antigen sequence necessary for the binding of the member of the binding pair or eliciting of an immune response against the antigen. In particular embodiments, the binding site or antigen amino acid sequence is at least 4 amino acids in length, or is at least 6, 8, 10, 15, or 20 amino acids in length. In other embodiments the binding site amino acid sequence is no more than 10, 15, 20, or 25 amino acids in length, or is 5-10, 5-15, 5-20, 10-15, 10-20 or 10-25 amino acids in length.

In addition, the total length of the CDR (*i.e.*, the combined length of the binding site sequence and the rest of the CDR sequence) should be of an appropriate number of amino acids to allow binding of the antibody to the antigen. CDRs have been observed to have a range of numbers of amino acid residues, and the observed size ranges for the CDRs (as denoted by the abbreviations indicated in figure 2) are provided in Table 1.

Table 1

CDR	Number of residues
L1	10-17
L2	7
L3	7-11
H1	5-7
H2	9-12
H3	2-25

(compiled from data in Kabat and Wu, 1971, *Ann. NY Acad. Sci.* 190:382-93)

While many CDR H3 regions are of 5-9 residue in length, certain CDR H3 regions have been observed that are much longer. In particular, a number of antiviral antibodies have heavy chain CDR H3 regions of 17-24 residues in length.

Accordingly, in specific embodiments of the invention, the CDR containing the binding site or antigen portion is within the size range provided for that particular CDR in Table 1, *i.e.*, if it is the first CDR of the light chain, L1, the CDR is 10 to 17 amino acid residues; if it is the second CDR of the light chain, L2, the CDR is 7 amino acid residues; if it is the third CDR of the light chain, L3, the CDR is 7 to 11 amino acid residues; if it is the

first CDR of the heavy chain, H1, the CDR is 5 to 7 amino acid residues; if it is the second CDR of the heavy chain, H2, the CDR is 9 to 12 amino acid residues; and if it is the third CDR of the heavy chain, H3, the CDR is 2 to 25 amino acid residues. In other specific embodiments, the CDR containing the binding site is 5-10, 5-15, 5-20, 11-15, 11-20, 11-25, or 16-25 amino acids in length. In other embodiments, the CDR containing the binding site is at least 5, 10, 15, or 20 amino acids or is no more than 10, 15, 20, 25, or 30 amino acids in length.

After constructing antibodies containing modified CDRs, the modified antibodies can be further altered and screened to select an antibody having higher affinity or specificity. Antibodies having higher affinity or specificity for the target antigen may be generated and selected by any method known in the art. For example, but not by way of limitation, the nucleic acid encoding the synthetic modified antibody can be mutagenized, either randomly, *i.e.*, by chemical or site-directed mutagenesis, or by making particular mutations at specific positions in the nucleic acid encoding the modified antibody, and then screening the antibodies exposed from the mutated nucleic acid molecules for binding affinity for the target antigen. Screening can be accomplished by testing the expressed antibody molecules individually or by screening a library of the mutated sequences, *e.g.*, by phage display techniques (see, *e.g.*, U. S. Patent Nos. 5,223,409; 5,403,484; and 5,571,698, all by Ladner et al; PCT Publication WO 92/01047 by McCafferty et al. or any other phage display technique known in the art).

In specific embodiments, the invention provides a functionally active fragment, derivative or analog of the modified immunoglobulin molecules of the invention. Functionally active means that the fragment, derivative or analog is able to elicit anti-anti-idiotype antibodies (*i.e.*, tertiary antibodies or Ab3 antibodies) that recognize the same antigen that the antibody from which the fragment, derivative or analog is derived recognized (*e.g.*, as determined by the methods described in Section 5.4, *infra*). Specifically, in a preferred embodiment, the antigenicity of the idiotype of the immunoglobulin molecule may be enhanced by deletion of framework and CDR sequences that are N-terminal to the particular CDR sequence that specifically recognizes the antigen. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR sequences can be used in binding assays with the antigen by any binding assay method known in the art. Accordingly, in a preferred embodiment, the invention includes modified immunoglobulin molecules that have one disulfide bond forming cysteine residue in a variable region domain replaced with an amino acid residue that does not contain a sulfhydryl group and in which a portion of that variable domain has been deleted N-terminal to the CDR sequence that recognizes the antigen.

Other embodiments of the invention include fragments of the modified antibodies of the invention such as. but not limited to, $F(ab')_2$ fragments, which contain the variable region, the light chain constant region and the CH1 domain of the heavy chain can be produced by pepsin digestion of the antibody molecule, and Fab fragments, which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. The invention also provides heavy chain and light chain dimers of the modified antibodies of the invention, or any minimal fragment thereof such as Fvs or single chain antibodies (SCAs) (*e.g.*, as described in U.S. Patent 4,946,778; Bird, 1988, Science 242:423-42; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54), or any other molecule with the same specificity as the modified antibody of the invention.

Techniques have been developed for the production of "chimeric antibodies" (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a constant domain from a human immunoglobulin, *e.g.*, humanized antibodies.

In a preferred embodiment, the modified immunoglobulin of the invention is a humanized antibody, more preferably an antibody having a variable domain in which the framework regions are from a human antibody and the CDRs are from an antibody of a non-human animal, preferably a mouse (see, International Patent Application No. PCT/GB8500392 by Neuberger et al. and Celltech Limited).

CDR grafting is another method of humanizing antibodies. It involves reshaping murine antibodies in order to transfer full antigen specificity and binding affinity to a human framework (Winter et al. U.S. Patent No. 5,225,539). CDR-grafted antibodies have been successfully constructed against various antigens, for example, antibodies against IL-2 receptor as described in Queen et al., 1989 (Proc. Natl. Acad. Sci. USA 86:10029); antibodies against cell surface receptors-CAMPATH as described in Riechmann et al. (1988, Nature, 332:323); antibodies against hepatitis B in Cole et al. (1991, Proc. Natl. Acad. Sci. USA 88:2869); as well as against viral antigens-respiratory syncitial virus in Tempest et al. (1991, Bio-Technology 9:267). CDR-grafted antibodies are generated in which the CDRs of the murine monoclonal antibody are grafted into a human antibody. Following grafting, most antibodies benefit from additional amino acid changes in the framework region to maintain affinity, presumably because framework residues are necessary to maintain CDR conformation, and some framework residues have been

demonstrated to be part of the antigen binding site. However, in order to preserve the framework region so as not to introduce any antigenic site, the sequence is compared with established germline sequences followed by computer modeling.

In other embodiments, the invention provides fusion proteins of the modified immunoglobulins of the invention (or functionally active fragments thereof), for example in which the modified immunoglobulin is fused via a covalent bond (*e.g.*, a peptide bond), at either the N-terminus or the C-terminus to an amino acid sequence of another protein (or portion thereof, preferably an at least 10, 20 or 50 amino acid portion of the protein) that is not the modified immunoglobulin. Preferably the modified immunoglobulin, or fragment thereof, is covalently linked to the other protein at the N-terminus of the constant domain. In preferred embodiments, the invention provides fusion proteins in which the modified immunoglobulin is covalently linked to IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, γ-interferon, MHC derived peptide, G-CSF, a porin, TNF, NK cell antigens, or cellular endocytosis receptor.

The modified immunoglobulins of the invention include analogs and derivatives that are either modified, *i.e*, by the covalent attachment of any type of molecule as long as such covalent attachment does not prevent the modified immunoglobulin from generating an anti-idiotypic response (*e.g.*, as determined by any of the methods described in Section 5.5, *infra*). For example, but not by way of limitation, the derivatives and analogs of the modified immunoglobulins include those that have been further modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the analog or derivative may contain one or more non-classical amino acids, *e.g.*, as listed above in this Section.

Methods of producing the modified immunoglobulins, and fragments, analogs, and derivatives thereof, are described in Section 5.4, *infra*.

5.2. CONTRACEPTIVE METHODS

The present invention provides methods of contraception by eliciting production of anti-idiotype antibodies and anti-anti-idiotype antibodies in a subject by the administration of a therapeutic (termed herein "Therapeutic"). Such Therapeutics include the modified immunoglobulins of the invention, and functionally active fragments, analogs, and derivatives thereof (*e.g.*, as described in Section 5.1, *supra*), and nucleic acids encoding the

modified antibodies of the invention, and functionally active fragments and derivatives thereof (*e.g.*, as described in Section 5.1, *supra*).

Generally, administration of products of a species origin or species reactivity that is the same species as that of the subject is preferred. Thus, in a preferred embodiment, the methods of the invention use a modified antibody that is derived from a human antibody; in other embodiments, the methods of the invention use a modified antibody that is derived from a chimeric or humanized antibody.

Specifically, vaccine compositions (*e.g.*, as described in Section 5.3, *infra*) containing the modified antibodies of the invention are administered to the subject to elicit the production of an antibody (*i.e.*, the anti-idiotype antibody or Ab2) that specifically recognizes the idiotype of the modified antibody, the Ab2, in turn, elicits the production anti-anti-idiotype antibodies (Ab3) that specifically recognize the idiotype of Ab2, such that these Ab3 antibodies have the same or similar binding specificity as the modified antibody.

The invention provides methods of administering the modified antibodies of the invention to elicit an anti-idiotype response, *i.e.*, to generate Ab2 and Ab3 type antibodies. Alternatively, the invention provides methods of administering the modified antibodies of the invention to one subject to generate Ab2 antibodies, isolating the Ab2 antibodies, and then administering the Ab2 antibodies to a second subject to generate Ab3 type antibodies in that second subject.

Accordingly, the invention provides a method of generating an anti-idiotype response in a subject comprising administering an amount of first immunoglobulin molecule (or functionally active fragment, analog, or derivative thereof) sufficient to induce an anti-idiotype response, said first immunoglobulin comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule being capable of immunospecifically binding an antigen, said one or more amino acid substitutions being the substitution of an amino acid residue that does not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule. In another embodiment, the method further provides isolating the anti-idiotype antibody that recognizes the idiotype of said second immunoglobulin molecule, and administering to a second subject the anti-idiotype antibody.

Modified immunoglobulins capable of inhibiting the gamete interaction *i.e.*, of eggs and sperm are preferably employed. The key to this method of contraception is to either immunologically regulate molecules involved in reproduction or to inhibit fertilization. Such contraceptive vaccines target reproductive hormone or receptor-specific antigens or gamete-specific antigens. The goal is to elicit an immune response which targets

reproductive hormones or receptors or native gamete molecules. In prefered embodiments, the vaccine targets sperm by eliciting production of antibodies that recognize sperm antigens.

Fertility can be suppressed by immunization against a reproductive hormone or receptor such as gonadotropin-releasing hormone, gonadotropins, prostaglandin F2 alpha, oxytocin and gonadotropin receptors.

Fertility can also be suppressed by immunization against gamete or embryonic antigens. Fertilization is mediated through specific molecules of the sperm and egg. In mammals, the sperm and egg interact at an egg-specific extracellular matrix, the zona pellucida (ZP), and the sperm plasma membrane (Gupta et al., 1997, Hum. Reprod. Update, 3(4):311-324). The zona pellucida comprises three glycoproteins ZP1, ZP2 and ZP3 (Kaul et al., 1997, Mol. Reprod. Dev. 47(2):140-147) which are target antigens for designing immunocontraceptives. Some of the sperm plasma membrane proteins which are useful as antigens for immunocontraception are PH-20 (Primakoff et al., 1997, Biol. Reprod., 56(5):1142-1146) and PH-30 (Kerr, Reprod. Fertil. Dev., 1995, 7(4):825-830). Other sperm proteins are SP-10 (Kurth et al., 1997, Biol. Reprod., 57(5):981-989) and SP-17 (Adoyo et al., 1997, Mol. Reprod. Dev., 47(1):66-71). Other gamete proteins include lactate dehydrogenase-C4 (LDH-C4) (Bradley et al., Reprod. Fertil. Dev., 9(1):111-116) and fertilization antigen-1 (FA-1) (Zhu and Naz, Proc. Natl. Acad. Sci. USA., 94(9):4704-4709).

In particular, the contraceptive methods of the invention involve administration of modified immunoglobulin molecules (or functionally active fragments, derivatives or an analog thereof, or nucleic acids encoding the same) derived from an immunoglobulin molecule that specifically recognizes a molecule or cell involved in reproductive function. In a specific embodiment, the contraceptive methods of the invention involve the administration of a modified immunoglobulin molecule that is derived from an antibody that is capable of immunospecifically binding to gonadotropin-releasing hormone, any gonadotropin, prostaglandin F2 alpha, oxytocin, gonadotropin receptors, gamete or embryonic antigens, sperm antigens, preferably SP-10. Other antigens include, but are not limited to, lactate dehydrogenase LDH-C4, SP-17, PH-20, FA-1, FA-2, PH-30, RSA, HAS-63, MSA-63, or zona pellucida proteins ZP1, ZP2, and ZP3 (see, e.g., Freemerman et al., 1993, Molecular Reproduction and Development 34:140-148; Herr et al., 1990, Biol. Reproduction 42:181-193; O'Hern et al., 1995, Biol. Reproduction 52:331-339; Anderson et al., 1986, J. Reprod. Immunol. 10:231-257; Wright et al., 1990, Biology of Reproduction 42:693-701; Lea et al., 1997, Fertility and Sterility 67:355-361; O'Hern et al., Elsevier Science Ltd. 16:1761-1766; Kerr, 1995, Reprod. Fertil. Dev. 7:825-830; Kaul et al., 1996,

Reprod. Fertil Dev. 50:127-134; Liu et al., 1990, Molecular Reproduction and Development 25:302-308; Bambra, 1992, Scand. J. Immunol. 11:118-122).

The invention also includes contraceptive methods whereby a modified immunoglobulin of the invention is administered in conjunction with use of another contraceptive method, such as, but not limited to, barrier methods such as the use of condoms or diaphragms or cervical caps, or intravaginal use of contraceptive compounds such as, but not limited to, non-oxynol-9, intrauterine devices, birth control pills or implants, etc.

The invention also includes administrations of anti-anti-idiotype antibodies against a modified immunoglobulin of the invention to acutely neutralize the contraceptive activity of the modified immunoglobulin.

The methods and vaccine compositions of the present invention may be used to elicit a humoral and/or a cell-mediated response against a modified immunoglobulin in a subject. In one specific embodiment, the methods and compositions of the invention elicit a humoral response in a subject. In another specific embodiment, the methods and compositions of the invention elicit a cell-mediated response in a subject. In a preferred embodiment, the methods and compositions of the invention elicit both a humoral and a cell-mediated response.

The subjects to which the present invention is applicable may be any mammalian or vertebrate species, which include, but are not limited to, cows, horses, sheep, pigs, fowl (*e.g.*, chickens), goats, cats, dogs, hamsters, mice, rats, monkeys, rabbits, chimpanzees, and humans. In a preferred embodiment, the subject is a human.

5.2.1. GENE THERAPY

Gene therapy may be used by administering a nucleic acid containing a nucleotide sequence encoding the modified immunoglobulin of the invention as a contraceptive. In this embodiment of the invention, the therapeutic nucleic acid encodes a sequence that produces intracellularly (without a leader sequence) or intercellularly (with a leader sequence), a modified immunoglobulin.

For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, *Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; Kriegler,

1990, *Gene Transfer and Expression. A Laboratory Manual*. Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds), 1994, *Current Protocols in Human Genetics*, John Wiley & Sons, NY).

In one aspect, the therapeutic nucleic acid comprises an expression vector that expresses the modified immunoglobulin molecule.

Delivery of the nucleic acid into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vector or a delivery complex, or indirect, in which case, cells are first transformed with the nucleic acid *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid is directly administered *in vivo*, where it is expressed to produce the antibodies. This can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by infection using a defective or attenuated retroviral or other viral vector (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in biopolymers (*e.g.*, poly-ß-1->4-N-acetylglucosamine polysaccharide; see U.S. Patent No. 5,635,493), encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see *e.g.*, Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432), etc. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, PCT Publications WO 92/06180 dated April 16, 1992 (Wu et al.); WO 92/22635 dated December 23, 1992 (Wilson et al.); WO92/20316 dated November 26, 1992 (Findeis et al.); WO93/14188 dated July 22, 1993 (Young). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra et al., 1989, *Nature* 342:435-438).

Alternatively, single chain antibodies, such as neutralizing antibodies, which bind to intracellular epitopes can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population by utilizing, for example, techniques such as

those described in Marasco et al. (Marasco et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:7889-7893). Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson. 1993, *Current Opinion in Genetics and Development* 3:499-503 present a review of adenovirus-based gene therapy. Bout et al., 1994, *Human Gene Therapy* 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., 1991, *Science* 252:431-434; Rosenfeld et al., 1992, *Cell* 68:143-155; and Mastrangeli et al., 1993, *J. Clin. Invest.* 91:225-234. Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, *Proc. Soc. Exp. Biol. Med.* 204:289-300).

The form and amount of therapeutic nucleic acid envisioned for use depends on the type of disease and the severity of its desired effect, patient state, etc., and can be determined by one skilled in the art.

5.3. VACCINE FORMULATIONS AND ADMINISTRATION

The invention also provides vaccine formulations containing Therapeutics of the invention, which vaccine formulations are suitable for administration to elicit a protective immune (humoral and/or cell mediated) response against certain antigens, *e.g.*, for the contraceptive uses described herein. .

Suitable preparations of such vaccines include injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection, may also be prepared. The preparation may also be emulsified, or the polypeptides encapsulated in liposomes. The active immunogenic ingredients are often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, buffered saline, dextrose, glycerol, ethanol, sterile isotonic aqueous buffer or the like and combinations thereof. In addition, if desired, the vaccine preparation may also include minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine.

Examples of adjuvants which may be effective, include, but are not limited to: aluminim hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine.

The effectiveness of an adjuvant may be determined by measuring the induction of anti-idiotype antibodies directed against the injected immunoglobulin formulated with the particular adjuvant.

The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is administered by injection, an ampoule of sterile diluent can be provided so that the ingredients may be mixed prior to administration.

In a specific embodiment, the lyophilized modified immunoglobulin of the invention is provided in a first container; a second container comprises diluent consisting of an aqueous solution of 50% glycerin, 0.25% phenol, and an antiseptic (*e.g.*, 0.005% brilliant green).

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the vaccine formulations of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Composition comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

The subject to which the vaccine is administered is preferably a mammal, most preferably a human, but can also be a non-human animal including but not limited to cows, horses, sheep, pigs, fowl (*e.g.*, chickens), goats, cats, dogs, hamsters, mice and rats.

Many methods may be used to introduce the vaccine formulations of the invention; these include but are not limited to oral, intracerebral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal routes, and via scarification

(scratching through the top layers of skin, *e.g.*, using a bifurcated needle) or any other standard routes of immunization. In a specific embodiment, scarification is employed.

The precise dose of the modified immunoglobulin molecule to be employed in the formulation will also depend on the route of administration, and the nature of the patient, and should be decided according to the judgment of the practitioner and each patient's circumstances according to standard clinical techniques. An effective immunizing amount is that amount sufficient to produce an immune response to the modified immunoglobulin molecule in the host (*i.e.*, an anti-idiotype reaction) to which the vaccine preparation is administered. Effective doses may also be extrapolated from dose-response curves derived from animal model test systems.

5.4. METHOD OF PRODUCING THE MODIFIED IMMUNOGLOBULINS

The modified immunoglobulins of the invention can be produced by any method known in the art for the synthesis of immunoglobulins, in particular, by chemical synthesis or by recombinant expression, and is preferably produced by recombinant expression techniques.

Recombinant expression of the modified immunoglobulin of the invention, or fragment, derivative or analog thereof, requires construction of a nucleic acid that encodes the modified immunoglobulin. If the nucleotide sequence of the modified immunoglobulin is known, a nucleic acid encoding the modified immunoglobulin may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier et al., 1994, *BioTechniques* 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the modified immunoglobulin, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR, *e.g.*, as exemplified in Section 6, *infra*.

Alternatively, the nucleic acid encoding the modified immunoglobulin may be generated from a nucleic acid encoding the immunoglobulin from which the modified immunoglobulin was derived. If a clone containing the nucleic acid encoding the particular immunoglobulin is not available, but the sequence of the immunoglobulin molecule is known, a nucleic acid encoding the immunoglobulin may be obtained from a suitable source (*e.g.*, an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the immunoglobulin) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by hybridization using an oligonucleotide probe specific for the particular gene sequence.

If an immunoglobulin molecule that specifically recognizes a particular antigen is not available (or a source for a cDNA library for cloning a nucleic acid encoding such an

immunoglobulin is not available), immunoglobulins specific for a particular antigen may be generated by any method known in the art, for example, by immunizing an animal, such as a rabbit, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies, *e.g.*, as described by Kohler and Milstein (1975, *Nature* 256:495-497) or, as described by Kozbon et al. (1983, *Immunology Today* 4:72) or Cole et al. (1985 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Alternatively, a clone encoding at least the Fab portion of the immunoglobulin can be obtained by screening Fab expression libraries (*e.g.*, as described in Huse et al., 1989, *Science* 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (see, *e.g.*, Clackson et al., 1991, Nature 352:624; Hane et al., 1997 Proc. Natl. Acad. Sci. USA 94:4937).

Once a nucleic acid encoding at least the variable domain of the immunoglobulin molecule is obtained, it may be introduced into any available cloning vector, and may be introduced into a vector containing the nucleotide sequence encoding the constant region of the immunoglobulin molecule (see, *e.g.*, PCT Publication WO 86/05807; PCT Publication WO 89/01036; U.S. Patent No. 5,122,464; and Bebbington, 1991, Methods in Enzymology 2:136-145). Vectors containing the complete light or heavy chain for co-expression with the nucleic acid to allow the expression of a complete antibody molecule are also available, see *Id.* Then, the nucleic acid encoding the immunoglobulin can be modified to introduce the nucleotide substitutions or deletion necessary to substitute (or delete) the one or more variable region cysteine residues participating in an intrachain disulfide bond with an amino acid residue that does not contain a sulfhydyl group, along with any other desired amino acid substitutions, deletions or insertions. Such modifications can be carried out by any method known in the art for the introduction of specific mutations or deletions in a nucleotide sequence, for example, but not limited to, chemical muagenesis, *in vitro* site directed mutagenesis (Hutchinson et al., 1978, *J. Biol. Chem.* 253:6551), PCR based methods, etc.

In addition, techniques developed for the production of chimeric antibodies (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can also be used. As described *supra*, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a constmat region derived from a human immunoglobulin, *e.g.*, humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,694,778; Bird, 1988, *Science* 242:423-42; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., 1988, *Science* 242:1038-1041).

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments.

Once a nucleic acid encoding the modified immunoglobulin molecule of the invention has been obtained, the vector for the production of the immunoglobulin molecule may be produced by recombinant DNA technology using techniques well known in the art. The modified immunoglobulin molecule can then be recombinantly expressed and isolated by any method known in the art, for example, using the method described in Section 6, *supra*, (see also Bebbington, 1991, Methods in Enzymology 2:136-145). Briefly, COS cells, or any other appropriate cultured cells, can be transiently or non-transiently transfected with the expression vector encoding the modified immunoglobulin, cultured for an appropriate period of time to permit immunoglobulin expression, and then the supernatan can be harvested from the COS cells, which supernatant contains the secreted, expressed modified immunoglobulin.

Methods which are well known to those skilled in the art can be used to construct expression vectors containing the immunoglobulin molecule coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. See, for example, the techniques described in Sambrook et al. (1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and Ausubel et al. (eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY).

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce the immunoglobulin of the invention.

The host cells used to express the recombinant antibody of the invention may be either bacterial cells such as *Escherichia coli*, or, preferably, eukaryotic cells, especially for

the expression of whole recombinant immunoglobulin molecules. In particular, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for immunoglobulins (Foecking et al., 198, *Gene* 45:101; Cockett et al., 1990, *Bio/Technology* 8:2).

A variety of host-expression vector systems may be utilized to express the modified immunoglobulin molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express the immunoglobulin molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (*e.g.*, *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing immunoglobulin coding sequences; yeast (*e.g.*, *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing immunoglobulin coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the immunoglobulin coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing immunoglobulin coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the immunoglobulin molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an immunoglobulin molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, *EMBO J.* 2:1791), in which the immunoglobulin coding sequence may be ligated individually into the vector in frame with the *lac Z* coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-

agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The immunoglobulin coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the immunoglobulin coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the immunoglobulin molecule in infected hosts (*e.g.*, see Logan & Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:355-359). Specific initiation signals may also be required for efficient translation of inserted immunoglobulin coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, *Methods in Enzymol.* 153:51-544).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the immunoglobulin molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the immunoglobulin molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the immunoglobulin molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., 1977, *Cell* 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 192, *Proc. Natl. Acad. Sci. USA* 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell* 22:817) genes can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., 1980, *Natl. Acad. Sci. USA* 77:357; O'Hare et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072); neo, which confers resistance to the aminoglycoside G-418Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11(5):155-215). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds), 1994, Current Protocols in Human Genetics, John Wiley & Sons, NY.; Colberre-Garapin et al., 1981, *J. Mol. Biol.* 150:1.

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:8972-897). In

this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+}-nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

The expression levels of the immunoglobulin molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, *the Use of Vectors Based on Gene Amplification for the Expression of Cloned Genes in Mammalian Cells in DNA Cloning*, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing immunoglobulin is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the immunoglobulin gene, production of the immunoglobulin will also increase (Crouse et al., 1983, *Mol. Cell. Biol.* 3:257).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, *Nature* 322:52; Kohler, 1980, *Proc. Natl. Acad. Sci. USA* 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once the modified immunoglobulin molecule of the invention has been recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

5.5. DEMONSTRATION OF THERAPEUTIC UTILITY

The modified antibodies of the invention can be screened or assayed in a variety of ways for efficacy in treating or preventing a particular disease .

First, the immunopotency of a vaccine formulation containing the modified antibody of the invention can be determined by monitoring the anti-idiotypic response of test animals following immunization with the vaccine. Generation of a humoral response may be taken

as an indication of a generalized immune response, other components of which, particularly cell-mediated immunity, may also be important. Test animals may include mice, rabbits, chimpanzees and eventually human subjects. A vaccine made in this invention can be made to infect chimpanzees experimentally. However, since chimpanzees are a protected species, the antibody response to a vaccine of the invention can first be studied in a number of smaller, less expensive animals, with the goal of finding one or two best candidate immunoglobulin molecules or best combinations of immunoglobulin molecules to use in chimpanzee efficacy studies.

The immune response of the test subjects can be analyzed by various approaches such as the reactivity of the resultant immune serum to antibodies, as assayed by known techniques, *e.g.*, enzyme linked immunosorbent assay (ELISA), immunoblots, radioimmunoprecipitations, etc.; or protection from infection and/or attenuation of disease symptoms in immunized hosts.

As one example of suitable animal testing, the vaccine composition of the invention may be tested in rabbits for the ability to induce an anti-idiotypic response to the modified immunoglobulin molecule. For example, male specific-pathogen-free (SPF) young adult New Zealand White rabbits may be used. The test group of rabbits each receives an effective amount of the vaccine. A control group of rabbits receives an injection in 1 mM Tris-HCl pH 9.0 of the vaccine containing a naturally occurring antibody. Blood samples may be drawn from the rabbits every one or two weeks, and serum analyzed for anti-idiotypic antibodies to the modified immunoglobulin molecule and anti-anti-idiotypic antibodies specific for the antigen against which the modified antibody was directed using, *e.g.*, a radioimmunoassay (Abbott Laboratories). The presence of anti-idiotypic antibodies may be assayed using an ELISA. Because rabbits may give a variable response due to their outbred nature, it may also be useful to test the vaccines in mice.

In addition, a modified antibody of the invention may be tested by first administering the modified antibody to a test subject, either animal or human, and then isolating the anti-anti-idiotypic antibodies (*i.e.*, the Ab3 antibodies) generated as part of the anti-idiotype response to the injected modified antibody. The isolated Ab3 may then be tested for the ability to bind the particular antigen (*e.g.*, a tumor antigen, antigen of an infectious disease agent by any immunoassays known in the art, for example, but not limited to, radioimmunoassays, ELISA, "sandwich" immunoassay, gel diffusion precipitin reactions, immunodiffusion assays, western blots, precipitation reactions, agglytination assays, complement fixation assays, immunofluorescence assays, protein A assays, immunoelectrophoresis assays, etc.

Additionally, the modified antibodies of the invention may also be tested directly *in vivo*. The strength of the immune response *in vivo* to the modified immunogluobulin may be determined by any method known in the art, for example, but not limited to, delayed hypersensitivity skin tests and assays of the activity of cytolytic T-lymphocytes *in vitro*.

Delayed hypersensitivity skin tests are of great value in the testing of the overall immunocompetence and cellular immunity to an antigen. Proper technique of skin testing requires that the antigens be stored sterile at 4°C, protected from light and reconstituted shortly before use. A 25- or 27-gauge need ensures intradermal, rather than subcutaneous, administration of antigen. Twenty-four and 48 hours after intradermal administration of the antigen, the largest dimensions of both erythema and induration are measured with a ruler. Hypoactivity to any given antigen or group of antigens is confirmed by testing with higher concentrations of antigen or, in ambiguous circumstances, by a repeat test with an intermediate test.

To test the activity of cytolytic T-lymphocytes, T-lymphocytes isolated from the immunized subject, *e.g.*, by the Ficoll-Hypaque centrifugation gradient technique, are restimulated with cells bearing the antigen against which the modified antibody was directed in 3 ml RPMI medium containing 10% fetal calf serum. In some experiments, 33% secondary mixed lymphocyte culture supernatant or IL-2 is included in the culture medium as a source of T cell growth factors. In order to measure the primary response of cytolytic T-lymphocytes after immunization, the isolated T cells are cultured with or without the cells bearing the antigen. After six days, the cultures are tested for cytotoxity in a 4 hour ^{51}Cr-release assay. The spontaneous ^{51}Cr-release of the targets should reach a level less than 20% if immunization was effective (Heike et al., *J. Immunotherapy* 15:15-174).

The efficacy of the modified antibody as a contraceptive can also be tested by any method known for tested contraceptive methods. For example, a vaccine composition containing a modified antibody of the invention specific for an antigen of a protein or cell involved in reproductive function. First, the level of the particular antigen in the subject can be measured by any method known in the art where a reduction in the level of the antigen compared to the level prior to administration of the modified antibody (accounting for normal, cyclical changes of the level of the particular antigen) indicates that the modified antibody may be effective. The modified antibody must then be administered to a population of child bearing age (and having partners of childbearing age) and the percentage of females that conceive over a suitable period of time is determined. If the number of females that conceive is significantly lower than those in a control population, *e.g.*, those administered a placebo or not using a contraceptive method, indicates that the modified antibody is effective as a contraceptive.

Additionally, the efficacy of the contraceptive vaccine may be assayed by administering the vaccine to a subject or animal model, allowing an appropriate amount of time for the production of anti-idiotype antibodies, and then testing serum taken from the subject or animal for the ability to bind the particular antigen (indicating that an anti-idiotype reaction has occurred) and/or testing whether the serum can block fertilization in vitro, which can be tested by any method known in the art, for example as described in Brannen-Brock et al., 1985, Arch. Androl. 15:15-19. .

6. EXAMPLE: ANTI-IDIOTYPIC VACCINE INDUCER FOR COLON CANCER

This example describes the construction of a modified antibody derived from the monoclonal antibody MAb31.1 (hybridoma secreting Mab31.1 is available from the American Type Tissue Collection as accession No. HB12314). Mab31.1 recognizes an antigen expressed by human colon carcinomas. The modified antibody of the invention, based on Mab31.1, was engineered to have variable region cysteine residues of both the heavy and light chain variable regions substituted with alanine. Therefore, the resulting modified antibody, was missing intrachain disulfide bonds in either the heavy and light chain variable regions.

6.1. CONSTRUCTION OF A MODIFIED ANTIBODY

The strategy for construction of the modified antibody was to construct two engineered genes that encoded the heavy and light chain variable regions wherein specific cysteine residues, known to be important in intra-chain disulfide bonding , were altered to alanine. Alanine residues were substituted for the cysteine residues at positions 22 and 92 of the heavy chain variable region of the antibody derived from Mab31.1 or at positions 23 and 88 of the Mab31.1 light chain variable region of the antibody derived from Mab31.1. In order to construct these engineered genes, groups of olionucleotides were assembled (as discussed below) and inserted into an appropriate vector providing constant regions.

In order to construct variable region genes encoding CDRs lacking intrachain disulfide bonds, the following strategy was performed.

First, single strand oligonucleotides were annealed to create cohesive double stranded DNA fragments (as diagramed in Figure 8, Step 1). Specifically, oligonucleotides of about 80 bases in length corresponding to the sequences of interest with 20 base overlapping regions were synthesized using automated techniques of GenoSys Biotech Inc. The specific sequences of each of these oligonucleotides. The specific sequences of these

oligonucleotides are presented in Figures 16A and 16B. Figure 16A list the group of ten oligos used in engineering a heavy chain variable region gene called 2CAVHCOL1. 2CAVHCOL1 lacked 2 cysteine residues as compared to the consensus heavy chain variable gene. Figure 16B lists the group of 12 oligos used in the engineering of the light chain variable region gene called 2CAVLCOL1. 2CAVLCOL1 lacked two cysteine residues as compared to the consensus light chain variable region gene. In order to combine the oligos into the desired gene, groups of 10 or 12 oligos were combined as described below and as presented in Figure 8, where the identities of oligos 1 to 10 indicated in Figure 8 are provided in Table 5. Prior to combining, each oligonucleotide was 5' phosphorylated as follows: 25µl of each oligo was incubated for 1 hour in the presence of T4 polynucleotide kinase and 50mM ATP at 37°C. The reactions were stopped by heating for 5 minutes at 70°C followed by ethanol precipitation. Once phosphorylated, complementary oligonucleotides (oligo 1 + oligo 10, oligo 2 + oligo 9, oligo 3 + oligo 8, oligo 4 + oligo 7, oligo 5 + oligo 6), as shown in Figure 8, were then mixed in sterile microcentrifuge tubes and annealed by heating the tube in a water bath at 65°C for 5 minutes followed by cooling at room temperature for 30 minutes. Annealing resulted in short double strand DNA fragments with cohesive ends.

Next, the cohesive double stand DNA fragments were ligated into longer strands (Figure 8, Steps 2-4), until the engineered variable region gene was assembled. Specifically, cohesive double strand DNA fragments were ligated in the presence of T4 DNA ligase and 10mM ATP for 2 hours in a water bath maintained at 16°C. Annealed oligo 1/10 was mixed with annealed oligo 2/9, and annealed oligo 3/8 was mixed with annealed oligo 4/7. The resulting oligos were labeled oligo 1/10/2/9 and oligo 3/8/4/7. Next, oligo 3/8/4/7 was ligated to oligo 5/6. The resulting oligo 3/8/4/7/5/6 was then ligated to oligo 1/10/2/9 resulted in a full length variable region gene.

Alternatively, when groups of 12 oligos were used, the order of addition was: 1+12 = 1/12, 2+11=2/11, 3+10=3/10, 4+9=4/9, 5+8=5/8, 6+7=6/7, 1/12+2/11=1/12/2/11, 3/10+4/9=3/10/4/9, 5/8+6/7=5/8/6/7, 1/12/2/11+3/10/4/9 = 1/12/2/11/3/10/4/9, 1/12/2/11/3/10/4/9+5/8/6/7= full length variable region gene. The names of oligonucleotides used in construction of the engineered genes are listed in Table 5. The modified heavy chain variable region gene was denoted as 2CAVHCOL1. The modified light chain variable region gene was denoted as 2CAVLCOL1.

The resulting modified variable region genes were then purified by gel electrophoresis. To remove unligated excess of oligos and other incomplete DNA fragments, ligated product was run on 1% low melting agarose gel at constant 110 V for 2 hours. The major band containing full length DNA product was cut out and placed in a

sterile 1.5 ml centrifuge tube. To release the DNA from the agarose, the gel slice was digested with f3-Agrase I at 40°C for 3 hours. The DNA was recovered by precipitation with 0.3 M NaOAc and isopropanol at —20°C for 1 hour followed by centrifugation at 12,000 rpm for 15 minutes. The purified DNA pellet was resuspended in 50 µl of TE buffer, pH 8.0. The engineered variable region gene was then amplified by PCR. Specifically, 100 ng of the engineered variable region gene was mixed with 25mM dNTPs, 200 ng of primers and 5 U of high fidelity thermostable Pfu DNA polymerase in buffer. Resulting PCR product was analyzed on 1% agarose gel.

Each purified DNA corresponding to the engineered variable region gene was subsequently inserted into the pUC19 bacterial vector. pUC19, is a 2686 base pair, a high copy number *E. coli* plasmid vector containing a 54 base pair polylinker cloning site in lacZ and an Amp selection marker. In order to prepare the vector for insertion of the engineered variable region gene, 10µg of pUC19 was linearized with *Hinc II* (50 U) for 3 hours at 37°C resulting in a vector with blunt end sequence 5' GTC. To prevent self re-ligation, linear vector DNA was dephosphorylated with 25 U of calf intestine alkaline phosphatase (CIP) for 1 hour at 37°C. In order to insert the engineered variable region gene into the pUC19 vector, approximately 0.5 µg of dephosphorylated linear vector DNA was mixed with 3 µg of phosphorylated variable region gene in the presence of T4 DNA ligase (1000 U), and incubated at 16°C for 12 hours.

The bacterial vector containing the engineered variable region gene was then used to transform bacterial cells. Specifically, freshly prepared competent DH5-α cells, 50 µl, were mixed with 1 µg of pUC19 containing the engineered variable region gene and transferred to an electroporation cuvette (0.2 cm gap; Bio-Rad). Each cuvette was pulsed at 2.5 kV/200 ohm/25 µF in an electroporator (Bio-Rad Gene Pulser). Immediately thereafter, 1 ml of SOC media was added to each cuvette and cells were allowed to recover for 1 hour at 37°C in centrifuge tubes. An aliquot of cells from each transformation was removed, diluted 1:100, then 100 µl plated onto LB plates containing ampicillin (Amp 40 µg/ml). The plates were incubated at 37°C overnight due to the presence of the Amp marker. Only transformants containing pUC19 vector grew on LB/Amp plates.

A single transformant colony was picked and grown overnight in a 3 ml LB/Amp sterile glass tube with constant shaking at 37°C. The plasmid DNA was isolated using Easy Prep columns (Pharmacia Biotech.) and suspended in 100 µl of TE buffer, pH 7.5. To confirm the presence of gene insert in pUC19, 25 µl of plasmid DNA from each colony was digested with a restriction endonuclease for 1 hour at 37°C, and was analyzed on a 1% agarose gel. By this method plasmid DNA containing gene insert was resistant to enzyme cleavage due to loss of restriction site (5'..GTCGAC.. 3') and migrated as closed circular

(CC) DNA, while those plasmids without insert were cleaved and migrated as linear (L) double strand DNA fragment on gel.

In order to confirm correct gene sequences of the engineered variable region genes and to eliminate the possibility of unwanted mutations generated during the construction procedure, DNA sequencing was performed using M13/pUC reverse primer (5'AACAGCTATGACCATG 3') for the clones as well as PCR gene products using 5' end 20 base primer (5' GAATT CATGGCTTG GGTGTG 3') on automated ABI 377 DNA Sequencer. All clones were confirmed to contain correct sequences.

Table 5. Construction of gene encoding modified antibodies containing CDRs from Mab 31.1

	Oligo1	Oligo 2	Oligo 3	Oligo 4	Oligo 5	Oligo	Oligo 7	Oligo 8	Oligo 9	Oligo10
2CAVHC OLI	VHC1	VHC2	VHC3	VHC4	VHC5	VHC	VHC7	VHC8	VHC9	VHC10
2CAVLC OLI	VLC1	VLC2	VLC3	VLC4	VLCS	VLC	VLC7	VLC8	VLC9	VLC10

6.3. INSERTION OF THE ENGINEERED VARIABLE REGION GENE INTO A MAMMALIAN EXPRESSION VECTOR

A complete antibody light chain has both a variable region and a constant region. A complete antibody heavy chain contains a variable region, a constant region, and a hinge region. A modified variable region genes 2CAVHCOL1 or 2CAVLCOL1 were inserted into vectors containing appropriate constant regions. Engineered variable region genes lacking cysteine residues in the light chain, were inserted into the pMRRO10.1 vector Figure 6A. The pMRRO10.1 vector contained a human kappa light chain constant region. Insertion of the engineered light chain variable region into this vector gave a complete light chain sequence. Alternatively, the engineered variable region gene lacking cysteine residues in the heavy chain, were inserted into the pGAMMA1 vector Figure 6B. The pGAMMA1 vector contained human and IgG1 constant region and hinge region sequences. Insertion of the engineered heavy chain variable region gene into this vector gave a complete heavy chain sequence.

In order to engineer a mammalian vector comprising both heavy chain and light chain genes, the complete light chain sequence and complete heavy chain sequence were inserted into mammalian expression vector pNEPuDGV as shown in Figure 6C (Bebbington, C.R.. 1991. In METHODS: A Companion to Methods in Enzymology, vol. 2, pp. 136-145). The resulting vector encoding both light chain and the heavy chain of the modified antibody.

6.4. EXPRESSION OF SYNTHETIC MODIFIED ANTIBODIES IN MAMMALIAN CELLS

To examine the production of assembled antibodies the mammalian expression vector was transfected into COS cells. COS cells (an African green monkey kidney cell line, CV-1, transformed with an origin-defective SV40 virus) were used for short-term transient expression of the synthetic antibodies because of their capacity to replicate circular plasmids containing an SV40 origin of replication to very high copy number. The antibody expression vector was transferred to COS7 cells (obtained from the American Type Culture Collection). The transfected cells were grown in Dulbecco's modified Eagle's Medium and transfected with the expression vectors using calcium precipitation (Sullivan et al., *FEBS Lett.* 285:120-123, 1991). The transfected cells were cultured for 72 hours after which supernatants were collected. Supernatants from transfected COS cells were assayed using ELISA method for assembled IgG. ELISA involves capture of the samples and standards onto a 96-well plate coated with an anti-human IgG Fc. Bound assembled IgG was detected with an anti-human Kappa chain linked to horse radish peroxidase (HRP) and the substrate tetramethylbenzidine (TMB). Color development was proportional to the amount of assembled antibody present in the sample.

6.5. MODIFIED ANTIBODY IMMUNOSPECIFICALLY BINDS TO HUMAN COLON CARCINOMA CELLS AND ANTIGENS PRODUCED BY THESE CELLS

The modified antibody was expressed and isolated as indicated in Section 6.4, *supra*. The binding capacity and specificity were then assayed using LS-174T cells WiDR cells (a human colon cancer cell line) and an antigen derived from these cells.

In order to examine the binding potency as well as specificity of MA31.1 binding, a dot blot analysis was performed (see Figure 9). Membrane preparations from LS-174T cells was applied to a nitrocellulose membrane using a Bio-Blot apparatus (Bio-Rad). The wells were blocked for non-specific binding using skim milk. Biotinylated antibody derived from Mab31.1 was incubated in all wells. Unlabelled antibody at concentrations of 0.003 to 20 nM was then applied to the nitrocellulose membrane and allowed to incubate. Unbound antibody was removed from the membrane by washing and a second antibody, alkaline phosphatase labeled antihuman IgG, was added. Finally, an alkaline phosphatase substrate was added which generated a dark purple precipitate, indicating the presence of bound labeled antibody. Figure 9 provides the results of the dot blot analysis. The figure demonstrated that the labeled antibody bound to the LS-174 T cells. Additionally, the unlabeled antibody competed with biotinylated antibody binding, indicating specificity of binding of the antibody derived from Mab31.1 to tumor cell antigens.

6.6. ANTI-IDIOTYPE RESPONSE

The effect on binding of modified antibody to LS-174T cells was examined in a competition binding assay. LS-174T cells are human colon carcinoma cells which express antigen recognized by the Mab31.1 antibody. Peptides containing the sequence of one of the CDRs of the Mab31.1 antibody were generated. These peptides, the modified antibody and the control antibody derived from Mab31.1 were administered to mice in order to generate antisera against the CDR regions of Mab31.1 and the antibodies. Blood samples from mice were drawn two weeks and four weeks following injection. Antisera from the immuized mice were used in binding competition assays presented in Figures 10A and B.

Antisera and biotinylated antibodies were assayed for their ability to bind LS-174T cells. As demonstrated in Figure 10A and B, antisera raised to the CDR3 and CDR4 peptides dramatically competed for control antibody (antibody derived from Mab31.1) binding to LS-174T cells. Additionally, antisera raised against CDR1 and CDR2 also significantly competed for control antibody binding to LS-174T cells. Additionally, antisera from nice injected with the 2CAVHCOL1 and 2CAVLCOL1 antibodies (*i.e.*, the modified antibodies having the cysteine to alanine change in the variable region) competed for binding with the biotinylated antibody derived from Mab31.1 better than antiserum from mice injected with the antibody derived from Mab31.1 (Figure 10B). This result indicates that administration of the antibodies having the cysteine to alanine change in the variable region elicit an anti-idiotype antibodies that recognize the colon carcinoma cell antigen better than antibodies with variable region intra-chain disulfide bonds.

Table 6. Biotin-Labeled Peptides Derived from CDR Sequences of Mab 31.1

Peptide ID	Sequence
COL311 L1	biotin-N-Thr-Ala-Lys-Ala-Ser-Gln-Ser-Val-Ser-Asn-Asp-Val-Ala
COL311 L2	biotin-N-Ile-Tyr-Tyr-Ala-Ser-Asn-Arg-Tyr-Thr
COL311 L3	biotin-N-Phe-Ala-Gln-Gln-Asp-Tyr-Ser-Ser-Pro-Leu-Thr
COL311 H1	biotin-N-Phe-Thr-Asn-Tyr-Gly-Met-Asn
COL311 H2	biotin-N-Ala-Gly-Trp-Ile-Asn-Thr-Tyr-Thr-Gly-Glu-Pro-Thr-Tyr-Ala-Asp-Asp-Phe-Lys-Gly
COL311 H3	biotin-N-Ala-Arg-Ala-Tyr-Tyr-Gly-Lys-Tyr-Phe-Asp-Tyr

7. EXAMPLE: SPERM ANTIGEN VACCINES

The example herein describes the construction of defined epitopes that replace the complementarity determining regions (CDR) of an antibody. Specifically, the

epitopes are derived from sperm antigens SP-10, LDH-C_4 or MSA-63. These constructs express an antibody, which, when injected into an appropriate host, induces an immune reaction that precipitates the formation of anti-idiotype antibodies that are active against the sperm antigens.

The strategy for producing the antibody containing a sperm cell epitope is outlined as follows: (1) a CDR is engineered to contain a nucleotide sequence encoding one or more epitopes from a sperm specific protein, (2) the engineered CDR is then cloned into a mammalian expression vector containing the appropriate heavy or light chain constant regions, (3) the vector is transfected into a cell that supports expression, proper folding and modification of functional antibodies, (4) the antibody is harvested from the supernatant and is confirmed for the epitope expression by standard assays (e.g. ELISA, western blot, etc.), and (5) the antibody is used as an immunogen in an appropriate host to generate anti-sperm antibodies, thereby inducing long lasting infertility.

7.1. CONSTRUCTION OF THE SPERM ANTIGEN VACCINE

The following describes the construction of a modified variable region gene containing at least one CDR that contains a sperm antigen epitope, *i.e.*, SP-10 or LDH-C_4 epitope and/or an MSA-63 epitope.

First, an epitope is chosen and defined so that oligonucleotides may be synthesized. In the following example, an SP-10 epitope from the sperm antigen SP-10 is used. SP-10 is a suitable epitope because it is expressed exclusively in sperm cells. It is also expressed on the surface of the membrane of the acrosome, thus, it is accessible to therapeutic antibodies. Other antibodies are produced that contain portions of the LDH-C_4 and MSA-63 antigens.

The nucleotide and protein sequences of the SP-10 epitope are:

GAA TTC CAG CCT TCA GGT GAA CAT GGC TCC GGT GAA CAG CCT TCT GGT GAG CAG GCC TCG GGT GAA CAG CCT TCA GGT GAG CAC GCT TCA GGG GAA CAG GCT TCA GGT GCA CCA ATT TCA AGC ACA TCT ACA GGC ACA ATA TTA AAT TGC TAC ACA TGT GCT TAT ATG AAT GAT CAA GGA AAA TGT CTT CGT GGA GAG GGA ACC TGC ATC ACT CAG AAT TC;

Gln Pro Ser Gly Glu His Gly Glu Gln Pro Ser Gly Glu Gln Ala Ser Gly Glu Gln Pro Ser gly Glu His Ala Ser Gly Glu Gln Ala Ser Gly Ala Gin Ile Ser Ser Thr Ser Thr Gly Thr Ile Leu Asn Cys Tyr Thr Cys Ala Tyr Met Asn Asp Gln Gly Lys Cys Leu Arg Gly Glu Gly Thr Cys Ile Thr Gln Asn.

The replacement of an antibody's CDR with another epitope is made easier by the fact that the variable region sequence of antibodies are relatively short, and are known. One is then able to synthetically generate a series of complementary oligonucleotides that, when annealed and ligated, reconstruct the entire coding region of

variable region portion of the gene. In this manner, the CDR is replaced with sequences of the epitope of interest, in this example, SP-10. The following is a list of the sequences of the oligonucleotides designed for cloning SP-10 epitopes into the CDR:

Oligo SP 1:
GAA TTC CAG CCT TCA GGT GAA CAT GGC TCC GGT GAA CAG CCT TCT GGT GAG CAG GCC TCG GGT GAA CAG CCT TAG,
Oligo SP 2:
GTG AGC ACG CTT CAG GGG AAC AGC CTT CAG GTG CAC CAA TTT CAA GCA CAT CTA CAG GCA CAA TAT TAA ATT GCT,
Oligo SP 3:
ACA CAT GTG CTT ATA TGA ATG ATC AAG GAA AAT GTC TTC GTG GAG AGG GAA CCT GCA TCA CTC AGA ATT C,
Oligo SP 3a(3Cys-> Ala):
ACA CAG CAG CTT ATA TGA ATG ATC AAG GAA AAG CAC TTC GTG GAG AGG GAA CCG CAA TCA CTC AGA ATT C,
Oligo SP 4:
GAA TTC TGA GTG ATG CAG GTT CCC TCT CCA CGA AGA CAT TTT CCT TGA TCA TTC ATA TAA GCA CAT GTG TAG CAA TTT A,
Oligo SP 4a (3Cys->Ala):
GAA TTC TGA GTG ATT GCG GTT CCC TCT CCA CGA AGT GCT TTT TGA TGA TCA TTC ATA TAA GCT GCT GTG TAG CAA TTT A,
Oligo SP 5:
ATA TTG TGC CTG TAG ATG TGC TTG AAA TTG GTG CAC CTG AAG CCT GTT CCC CTG AAG CGT GCT CAC CTG AAG GCT,
Oligo SP 6:
GTT CTC CCG AGG CCT GCT CAC CAG AAG GCT GTT CAC CGG AGC CAT GTT CAC CTG AAG GCT GGA ATT C.

Antibodies containing portions of the MSA-63 antigen are also described. To identify the optimal portion of the antigen to be introduced into the antibody, oligonucleotides encoding different portions of the antigen are synthesized.

Practically, the first two amino acid codons of the sperm cell specific epitope, MSA-63, an oligonucleotide encoding residues 143 and 144 (i.e. GTC GGC, *infra*), is cloned into the immunoglobulin CDR, using the methods described *infra*,. The MSA-63 DNA sequence encoding the epitope:

GTC GGC AGC CTC CGA AGC AGC CCG CTC CAG AGC CCG CTG CTC CGA CCG CTC GTC CAG AGC AGC CTC TGC TTG CTG TTC CTC TTG CTG CGA TAC AGC TGC GGC GAC GGC AGC TGC AGC CGA CGA TAC TGC GAC TTG ACG GTG TGC CGG CGA ATG TAC TTG CTG CTG CGA TTC ACG GAC CCG CCG CTC CCG CAG ACG TGC TGC GTC TTG AGC

The MSA-63 protein sequence epitope encoded by the nucleic acid sequence above, which starts at amino acid 143 and ends at 233.

Gln Pro Ser Glu Ala Ser Ser Gly Glu Val Ser Gly Asp Glu Ala Gly Glu Gln Val Ser Ser Glu Thr Asn Asp Lys Glu Asn Asp Ala Met Ser Thr Pro Leu Pro Ser Thr Ser Ala Ala Ile

Thr Leu Asn Cys His Thr Cys Ala Tyr Met Asn Asp Asp Ala Lys Cys Leu Arg Gly Glu Gly Val Cys Thr Thr Gln Asn Ser

For the second two amino acid codons, an oligonucleotide encoding residues 144 and 145 is utilized (*i.e.*, GGC AGC). For the third, 145 and 146 and so on until the entire epitope is synthesized and inserted into the CDR, two amino acids at a time. For peptides three amino acids in length, an oligonucleotide encoding residues 143 to 146 is synthesized. The second oligonucleotide synthesized encodes residues 146 to 148. The third encodes residues 148 to 150, and this continues until the entire epitope is covered in this fashion. The next oligonucleotide that is synthesized is four amino acid codons in length. It begins with residues 143 to 146, its second segment is equivalent to residues 145 to 148, its third segment corresponds to residues 147 to 150, and so on until the entire epitope is transitioned in this fashion. The next oligonucleotide synthesized contains five amino acid codons with two overlapping with the previous. For example, the first oligonucleotide encodes residues 143 to 147, and the second residues 146 to 150. This pattern continues until the entire epitope has been transitioned. The next construct encoding an epitope uses nucleotides for six amino acid codons with two overlapping with the previous codons as described *infra*.

The epitopes thereafter contain peptides of seven residues with three overlapping. The pattern of adding one amino acid to each small peptide and increasing the overlap by one codon continues until an overlap of five is reached and then the small peptides are synthesized adding one codon each time until the full length of the epitope is encoded in the CDR. The overlap is never bigger than five amino acid codons although the entire peptide is lengthened by one amino acid in each new combination.

In a specific example, oligomers have been designed which scan the entire length of the MSA-63 epitope and encode 15 amino acids. Each oligo overlaps with the previous one for the equivalent of five amino acids. MSA-63 oligos encoding 15 amino acids, with overlap of five amino acids each:

MSA1: GTC GGC AGC CTC CGA AGC AGC CCG CTC CAG AGC CCG CTG CTC CGA

MSA2: AGC CCG CTG CTC CGA CCG CTC GTC CAG AGC AGC CTC TGC TTG CTG

MSA3: AGC CTC TGC TTG CTG TTC CTC TTG CTG CGA TAC AGC TGC GGC GAC

MSA4: TAC AGC TGC GGC GAC GGC AGC TGC AGC CGA CGA TAC TGC GAC TTG

MSA5: CGA TAC TGC GAC TTG ACG GTG TGC ACG CGA ATG TAC TTG CTG CTG

MSA6: ATG TAC TTG CTG CTG CGA TTC ACG GAC GCG CCG CTC CCG CAG ACG

MSA7: CGA TTC ACG GAC GCG CCG CTC CCG CAG ACG TGC TGC GTC TTG AGC

Antibodies in which a portion of the MSA-63 antigen has been inserted into CDR1, *i.e.*, where residues Lys24 through Ala34 of the consensus contraceptive light chain, the sequence of which is in Figure 15, are replaced with the sequence Gln-Pro-Ser-Glu-Ala-Ser-Ser-Gly-Glu-Val-Ser-Gly-Asp-Glu-Ala-Gly-Glu. The antibody, MSA1, can be constructed using the oligonucleotides provided in Figure 11 in the scheme provided in Figure 8 and described below, where the identities of oligonucleotides 1-12 are indicated in Table 7. The antibody MSA1VAC can also be constructed using the oligos of Figure 11 by the scheme of Figure 8, as indicated in Table 7. MSA1VAC is the same as MSA1 except that the cysteine at position 23 of the light chain variable region has been replaced with alanine. These light chains can be expressed with the heavy chain consensus sequence CONVH1, the sequence of which is provided in Figure 7B, and the construction of which can be accomplished with the oligonucleotides as indicated in Table 4. These single stranded oligonucleotides sequences are annealed to create cohesive double stranded DNA fragments suitable for ligation as diagramed in Figure 8, along with oligonucleotides encoding the remainder of the consensus variable region, to construct the variable region gene. For the MSA-63 containing variable regions MSA1 and MSA1VAC the oligonucleotides corresponding to oligonucleotides 1 to 10, or 1 to 12, of Figure 8 are provided in Table 7, and the sequences of these oligonucleotides are provided in Figure 11. Specifically, oligonucleotides of about 70 bases in length corresponding to the sequences of interest with 20 base overlapping regions are synthesized (GenoSys Biotech Inc.). Each oligonucleotide is 5' phosphorylated as follows: 25µl of each oligo is incubated for one hour in the presence of T_4 polynucleotide kinase and 50 mM ATP in appropriate buffer at 37°C. The enzyme is heat killed and the reaction stopped by heating for ten minutes at 70°C followed by ethanol precipitation with sodium acetate. The oligos are then resuspended in TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA).

Complementary oligonucleotides (oligo 1 + oligo 10, oligo 2 + oligo 9, oligo 3 + oligo 8, oligo 4 + oligo 7, and oligo 5 + oligo 6) were then mixed in a sterile microcentrifuge tube and annealed by heating the tube in a water bath at 65°C for 5 minutes followed by cooling at room temperature for 30 minutes. Annealing results in double stranded DNA with cohesive ends. The cohesive double stranded DNA fragments are ligated into longer strands (Figure 8, Steps 2-4), until the engineered variable region gene was assembled. Specifically, cohesive double stranded DNA fragments are ligated in the presence of T_4 DNA ligase, ligase buffer and 10 mM ATP for two hours in a water bath maintained at 16°C. Annealed oligo 1/10 is mixed with annealed oligo 2/9, and annealed oligo 3/8 is mixed with annealed oligo 4/7. The resulting oligos are 1/10/2/9 and 3/8/4/7. Next, oligo 3/8/4/7 is ligated to oligo 5/6. The resulting oligo 3/8/4/7/5/6 is then ligated to

oligo 1/10/2/9 resulting in a full length variable region gene. Alternatively, when 12 oligos are used, the order of addition is 1+12=1/12, 2+11=2/11, 3+10=3/10, 4+9=4/9, 5+8=5/8, 6+7=6/7, 1/12+2/11=1/12/2/11, 3/10+4/9=3/10/4/9, 5/8+6/7=5/8/6/7, 1/12/2/11+3/10/4/9=1/12/2/11/3/10/4/9, 1/12/2/11/3/10/4/9+5/8/6/7=1/12/2/11/3/10/4/9/5/8/6/7, which is the full length modified variable region gene. The names of oligonucleotides used for construction are listed in Table 7 and Figures 9, , 11, 12C, or 13C.

Using this method, variable region sequences in which an alanine has been substituted for a cysteine that forms an intrachain disulfide bond can be constructed using oligonucleotides introducing this change. For example, in constructing the antibody contains the SP-10 portion, oligos SP 3a and SP 4a could be used instead of oligo SP3 or SP4.

The modified variable region DNA fragment is then cloned into a shuttle vector (e.g. pUC19, *infra*) for sequence analysis and upon sequence confirmation, cloned into an expression vector. After running the DNA for two hours at 110 volts in a 1% low melting agarose gel, DNA fragments are visualized by ethidium bromide staining and gel slices are cut out and placed in a sterile microfuge tube. Gel purification removes excess free oligomers that may interfere with future ligations. The DNA is eluted from the agarose by addition with β-Agrase I at 40°C for three hours. DNA is precipitated using 0.3 M sodium acetate and isopropanol at -20°C for one hour, followed by centrifugation at high speed in a microcentrifuge for ten minutes. Isopropanol is aspirated and the pellet is washed once with 70% ethanol, the sample is spun again and the ethanol is aspirated and the pellet air dried. The DNA pellet is quantitated by running a small fraction of the resuspended pellet (i.e. 1/10th) on a gel and visually comparing to known DNA standards, or measuring the absorbance of UV light at 260 nM. If the quantity of DNA is to limiting for cloning at this point, it can be amplified by PCR techniques well known to those skilled in the art.

7.2 LIGATION OF THE MODIFIED CDR INTO PUC19

Purified DNA corresponding to the engineered variable region gene is subsequently inserted into the pUC19 vector by ligation. The pUC19 vector is a 2686 base

Table 7

	Oligo 1	Oligo 2	Oligo 3	Oligo 4	Oligo 5	Oligo 6	Oligo 7	Oligo 8	Oligo 9	Oligo 10	Oligo 11	Oligo 12
MSA 1	LDR	DSABL-1	MSAL-CDR1-1	HMVL3	HMVL4	HMVL6	HMVL6	HMVL7	HMVL8	MSAL-CDR1-1c	DSABL-1c	ANTIL DR
MSA1VAC	LDR	DSABL-1	MSALVAC-CDR1-1	HMVL3	HMVL4	HMVL5	HMVL6	HMVL7	HMVL8	MSALVAC-CDR1-1	DSABL-1c	ANTIL DR
ConVH1	BKHC1	BKHC2	BKHC3	BKHC4	BKHC5	BKHC6	BKHC7	BKHC8	BKHC9	BKHC10		

pair, high copy number *E. coli* plasmid containing a 54 base pair polylinker cloning site in the middle of the lacZ gene. The pUC19 vector also contains an ampicillin resistance marker for selection of bacteria containing the plasmid. The pUC19 is digested with the restriction enzyme *Hinc II* (10 μg plasmid in 50 units enzyme). The resulting blunt ends are dephosphorylated with calf intestinal phosphatase (CIP, 2 units in alkaline buffer, 30 minutes at 37°C), to prevent recircularization during the ligation step. The phosphatase is removed by extraction with phenol and chloroform, followed by precipitation with sodium acetate and ethanol on ice for 1 hour. The precipitated DNA is pelletted by high speed centrifugation and the ethanol is removed by aspiration, followed by a washing step with 70% ethanol to remove excess salts. The DNA pellet is air dried to completely remove any ethanol. The digested, phosphatased vector is then resuspended in TE buffer to 0.5 μg/μl. Approximately 0.1-0.5 μg of vector is incubated with a ten fold molar excess of the constructed variable region containing the sperm cell epitope in the CDR (modified variable region) with T_4 ligase (1000 units) in appropriate buffer and incubated at 16°C for 12 hours.

7.3 BACTERIAL TRANSFORMATION

The ligation mixture containing the engineered variable region gene cloned into pUC19, is transformed into competent bacterial cells. Specifically, 50 μl of freshly prepared competent DH5-α cells are mixed with the ligation mixture of pUC19 and modified variable region DNA and transferred to an electroporation cuvette (0.2 cm gap; Bio-Rad). Each cuvette is pulsed at 2.5 kV/200 ohm/25 μF in an electroporator (Bio-Rad Gene Pulser). Immediately thereafter, 1 ml of SOC media is added to each cuvette and cells are allowed to recover for 1 hour at 37°C in centrifuge tubes. An aliquot of cells from each transformation is removed, diluted 1:100, then 100 μl is plated onto LB plates with ampicillin (Amp 40 μg/ml). The plates are then incubated at 37°C overnight and only cells containing a plasmid grow.

The plasmid DNA is analyzed after isolation from single colonies picked by sterile toothpick and grown up overnight in 3 ml LB/Amp in a sterile glass test tube, with constant shaking at 37°C. The plasmid DNA is isolated using Easy Prep columns (Pharmacia Biotech) and suspended in 100 μl of TE buffer. To confirm the presence of insert, isolated plasmid DNA is digested with *Hinc II* and the digestion product is analyzed by 1.2% agarose gel electrophoresis in Tris-Acetate EDTA buffer (TAE). DNA is stained in the gel with ethidium bromide and visualized under UV light. The colonies that correspond to plasmids with insert are selected for further analysis.

7.4 DNA SEQUENCING

DNA sequencing is performed to verify the accuracy of the sequence in the cloned fragment. Sequencing across the pUC19 polylinker is done using the M13/pUC universal forward and universal reverse primers using the Sanger dideoxy chain termination procedure. The M13/pUC universal primers are readily found in biotechnology supply catalogues. Sequencing is performed on the ABI377 DNA sequencer, and sequence comparison is performed using standard computer alignment programs or visual inspection.

7.5 CLONING INTO THE V_H AND V_L CHAIN CONSTRUCTS

Once the sequence of the modified CDR has been confirmed, it is cut out of the pUC19 plasmid and ligated into either the heavy or light chain antibody expression vectors pMRRO10.1 or pGAMMA1, respectively (See Figures 6A and B). Alternatively, both the heavy and light chain genes are expressed on the same plasmid, and the modified CDR is ligated into either the heavy or light chain variable region as appropriate.

A complete antibody light chain has both a variable region and a constant region. A complete antibody heavy chain contains a variable region, a constant region, and a hinge region. The synthetic variable region genes of the invention are inserted into vectors containing appropriate constant regions. Engineered variable region genes with the sperm antigen epitope sequences are cloned into the pMRRO10.1 vector. The pMRRO10.1 vector contains a human kappa light chain constant region. Insertion of the engineered light chain variable region into this vector gives a complete light chain sequence. Alternatively, the engineered variable region gene with the sperm antigen sequence, of the heavy chain is inserted into the pGAMMA1 vector. The pGAMMA1 vector contains human and IgG1 constant region and hinge region sequences. Insertion of the engineered heavy chain variable region gene into this vector gave a complete heavey chain sequence.

In order to engineer a mammalian vector comprising both heavy chain and light chain genes, the complete light chain sequence and heavy chain sequence were inserted into a mammalian expression vector pNEPuDGV (Figure 6C; Bebbington, C., 1991, In METHODS: A Companion to Methods in Enzymology, 2:136-145). The resulting vector encodes both light chain and the heavy chain of the antibody.

7.6 TRANSFECTION OF EUKARYOTIC CELLS

The antibody expression plasmid, pNEPuDGV, is then transfected into a suitable host cell for expression of the antibody of interest. COS-7 (an African green monkey kidney cell line, CV-1, transformed with an origin defective SV40 virus), 293, or CHO cells are capable of being transfected and support expression of foreign proteins.

Transfection is performed by standard calcium phosphate precipitation (Sullivan et al., 1991, FEBS Lett. 285:120-123). Alternatively, cells may be transfected using lipid vesicles or electroporation. Transient or stable transfections are suitable depending on how much protein is expressed and harvested.

7.7 EXPRESSION AND PROTEIN ANALYSIS

Transfected cell supernatants are collected and analyzed for proper expression of anti-idiotype antibodies. The antibodies are purified away from cell debris and growth media serum and also concentrated from the supernatant by binding the antibody Fc domain to a protein A or protein G column. The antibody is eluted from the column by low pH glycine and dialyzed against BSA and Tris buffer.

7.8 IN VIVO ANALYSIS OF ANTI-IDIOTYPE EFFICACY

To test the ability of the antibody to elicit an immune response or for a contraceptive effect, the antibody is injected into a mouse at a pharmaceutically significant dose range and serum samples are taken from the mice. The production of anti-idiotype antibodies is confirmed by harvesting peripheral blood serum and performing ELISAs with the sperm antigen (or sperm), or western blots using the sperm antigen (or sperm) as target and the vaccinated mouse serum as probe.

ELISA involves capture of the samples and standards onto a 96 well plate coated with an anti-epitope antibody. Bound antibody is detected with a secondary antibody crosslinked to horse radish peroxidase (HRP) and the substrate tetramethylbenzidine (TMB) and specific to the kappa or lambda light chain of the mouse. Alternatively, western blots are performed using the anti-idiotype as the target and probing it with anti-epitope antibodies.

Confirmation of production of anti-idiotypes in the mice is then followed by *in vivo* analysis to determine whether the mice are capable of conception. Control mice and test mice are mated in statistically significant groups and the number of pregnancies are monitored. Effective immunocontraceptive therapy will result in a significant reduction in the number of pregnancies.

Additionally, the induction of effective quantities of anti-idiotype anti-bodies is also assayed for prevention of *in vitro* fertilization. Donor sperm is mixed in vitro with donor eggs in the presence or absence of test serum or negative control serum. The failure of sperm to fertilize the egg when test serum is added is a positive indication that the vaccine is effective.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. A vaccine composition comprising an amount of a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said first immunoglobulin molecule comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule having at least one complementarity determining region (CDR) that has a portion of an antigen of a cell or protein involved in reproductive function, said one or more amino acid substitutions being the substitution of one or more amino acid residues that do not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule; and a pharmaceutically acceptable carrier.

2. The vaccine composition according to claim 1, wherein said antigen is a sperm antigen.

3. The vaccine composition according to claim 2, wherein said sperm antigen is SP-10, MSA-63 or LDH-C4.

4. The vaccine composition according to claim 1, wherein said antigen is selected from the group consisting of gonadotropin-releasing hormone, a gonadotropin, prostaglandin F2 alpha, oxytocin, gonadotropin receptors, SP-17, PH-20, FA-1, FA-2, PH-30, RSA, HAS-63, ZP1, ZP2, and ZP3.

5. The vaccine composition according to claim 1, wherein a first CDR contains a portion of an antigen of a cell or protein associated with reproductive function and a second CDR contains a portion of an antigen of a cell or protein associated with reproductive function.

6. The vaccine composition according to claim 5, wherein said first CDR contains a portion of SP-10 antigen, and said second CDR contains a portion of LDH-C4.

7. The vaccine composition according to claim 1, wherein said variable region is a light chain variable region and said amino acid residue that does not have sulfhydryl group is at a position corresponding to position 23 or 88 in said light chain variable regionof said second immunoglobulin molecule.

8. The vaccine composition according to claim 1. wherein said variable region is a heavy chain variable region and said amino acid residue that does not have a sulfhydryl group is at a position corresponding to position 22 or 92 in said heavy chain variable region of said second immunoglobulin molecule.

9. The vaccine composition according to claim 1, 7 or 8, wherein said amino acid residue is alanine.

10. The vaccine composition according to claim 1, in which said first immunoglobulin molecule is of a type selected from the group consisting of IgG, IgE, IgM, IgD and IgA.

11. A vaccine composition comprising an amount of a fragment of a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said first immunoglobulin molecule comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule having at least one complementarity determining region (CDR) that has a portion of an antigen of a cell or protein involved in reproductive function, said one or more amino acid substitutions being the substitution of one or more amino acid residues that do not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule; and a pharmaceutically acceptable carrier.

12. The vaccine composition according to claim 11, wherein said antigen is a sperm antigen.

13. The vaccine composition according to claim 12, wherein said sperm antigen is SP-10, MSA-63 or LDH-C4.

14. The vaccine composition according to claim 11, wherein said antigen is selected from the group consisting of gonadotropin-releasing hormone, a gonadotropin, prostaglandin F2 alpha, oxytocin, gonadotropin receptors, SP-17, PH-20, FA-1, FA-2, PH-30, RSA, HAS-63, ZP1, ZP2, and ZP3.

15. The vaccine composition according to claim 11, wherein a first CDR contains a portion of an antigen of a cell or protein associated with reproductive function

and a second CDR contains a portion of an antigen of a cell or protein associated with reproductive function.

16. The vaccine composition according to claim 15, wherein said first CDR contains a portion of SP-10 antigen, and said second CDR contains a portion of LDH-C4.

17. The vaccine composition according to claim 11, wherein said variable region is a light chain variable region and said amino acid residue that does not have sulfhydryl group is at a position corresponding to position 23 or 88 in said light chain variable regionof said second immunoglobulin molecule.

18. The vaccine composition according to claim 11, wherein said variable region is a heavy chain variable region and said amino acid residue that does not have a sulfhydryl group is at a position corresponding to position 22 or 92 in said heavy chain variable region of said second immunoglobulin molecule.

19. The vaccine composition according to claim 11, 17 or 18, wherein said amino acid residue is alanine.

20. The vaccine composition according to claim 11, in which said first immunoglobulin molecule is of a type selected from the group consisting of IgG, IgE, IgM, IgD and IgA.

21. A method of contraception in a subject comprising administering to said subject an amount of a first immunoglobulin molecule sufficient to induce an anti-idiotype response, said first immunoglobulin molecule comprising a variable region and being identical, except for one or more amino acid substitutions in said variable region, to a second immunoglobulin molecule, said second immunoglobulin molecule having at least one complementarity determining region (CDR) that has a portion of an antigen of a cell or protein involved in reproductive function, said one or more amino acid substitutions being the substitution of one or more amino acid residues that do not have a sulfhydryl group at one or more positions corresponding to one or more cysteine residues that form a disulfide bond in said second immunoglobulin molecule.

22. The method according to claim 21 which further comprises isolating an antibody from said subject, said antibody recognizing the idiotype of said second immunoglobulin molecule and administering said antibody to a second subject.

23. The method according to claim 21, wherein said antigen is a sperm antigen.

24. The method according to claim 23, wherein said sperm antigen is SP-10, MSA-63 or LDH-C4.

25. The method according to claim 21, wherein said antigen is selected from the group consisting of gonadotropin-releasing hormone, a gonadotropin, prostaglandin F2 alpha, oxytocin, gonadotropin receptors, SP-17, PH-20, FA-1, FA-2, PH-30, RSA, HAS-63, ZP1, ZP2, and ZP3.

26. The method according to claim 21, wherein a first CDR contains a portion of an antigen of a cell or protein associated with reproductive function and a second CDR contains a portion of an antigen of a cell or protein associated with reproductive function.

27. The method according to claim 26, wherein said first CDR contains a portion of SP-10 antigen, and said second CDR contains a portion of LDH-C4.

28. The method according to claim 21, wherein said variable region is a light chain variable region and said amino acid residue that does not have sulfhydryl group is at a position corresponding to position 23 or 88 in said light chain variable regionof said second immunoglobulin molecule.

29. The method according to claim 21, wherein said variable region is a heavy chain variable region and said amino acid residue that does not have a sulfhydryl group is at a position corresponding to position 22 or 92 in said heavy chain variable region of said second immunoglobulin molecule.

30. The method according to claim 21, 28 or 29, wherein said amino acid residue is alanine.

31. The method according to claim 21, in which said first immunoglobulin molecule is of a type selected from the group consisting of IgG, IgE, IgM, IgD and IgA.

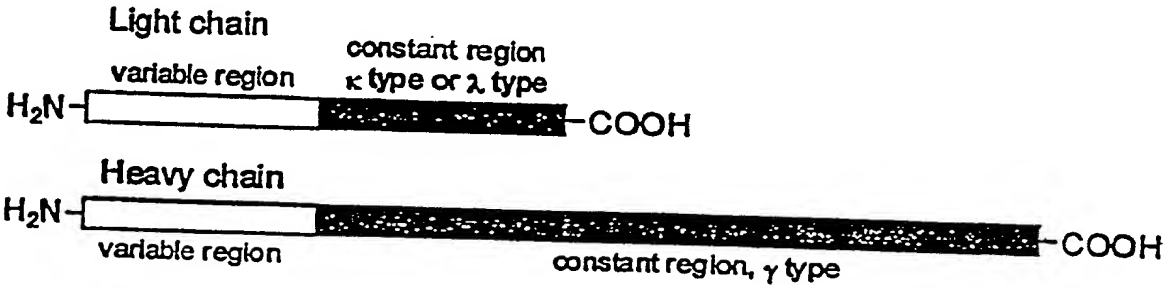

Light chain
variable region
constant region
κ type or λ type
H2N
COOH
Heavy chain
H2N
COOH
variable region
constant region, γ type

FIG. 1

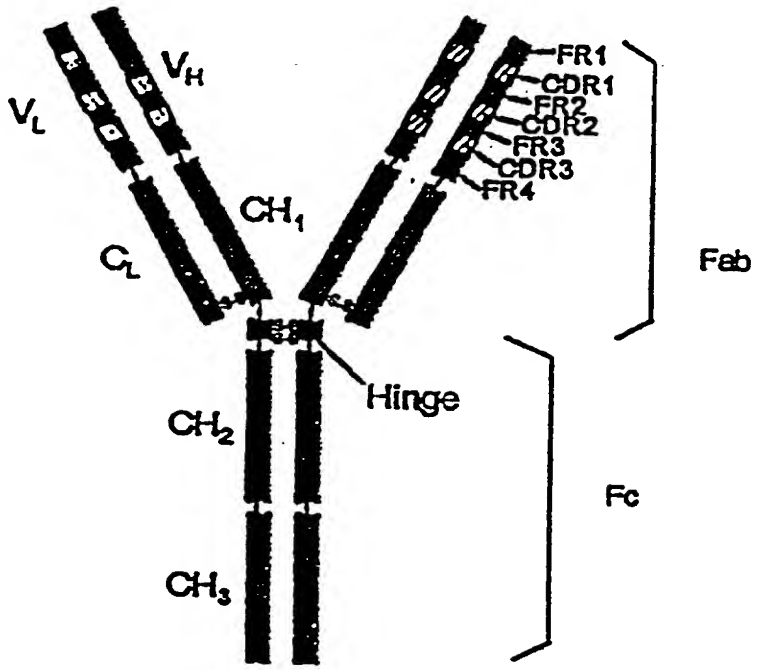


FIG. 2

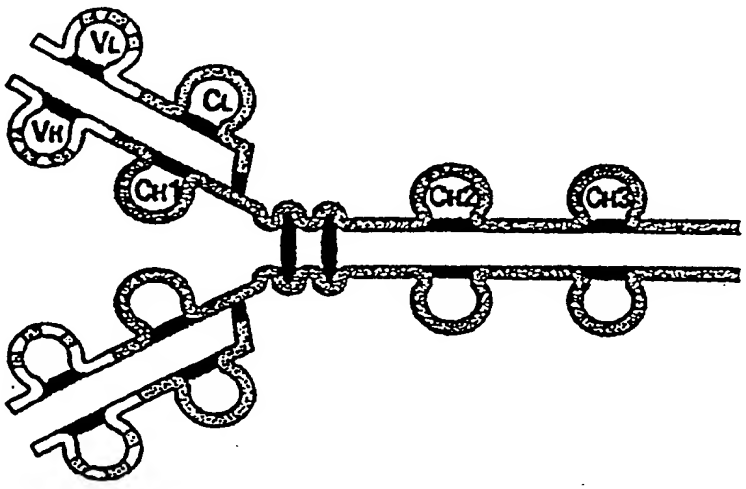


FIG. 3

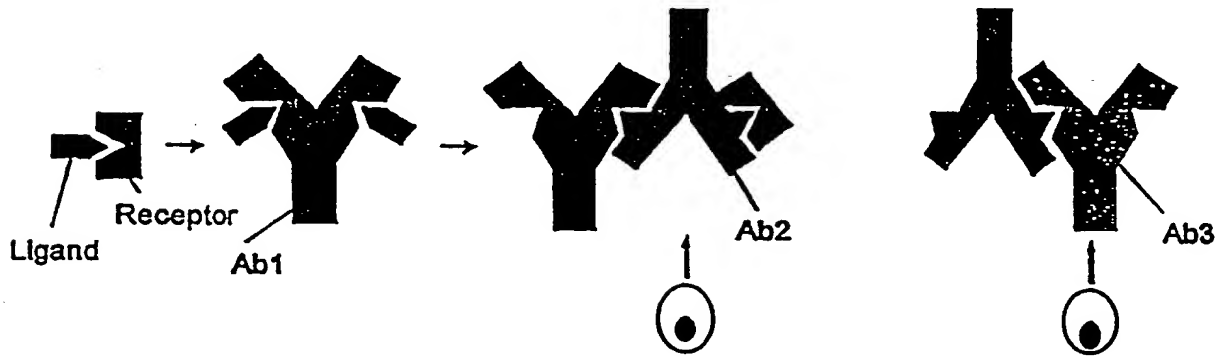


FIG. 4

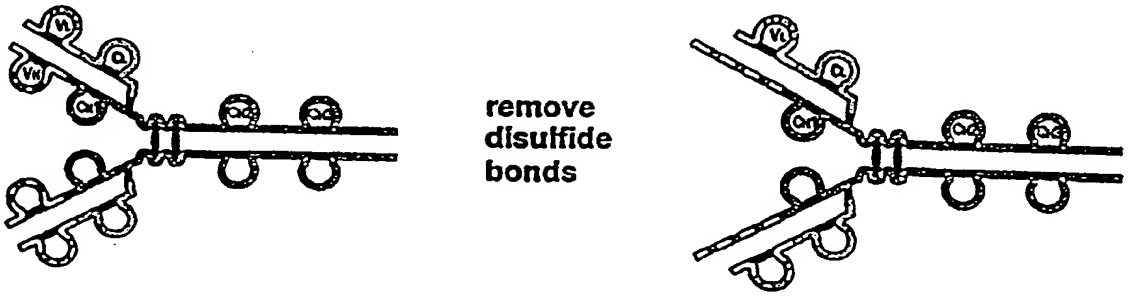


FIG. 5

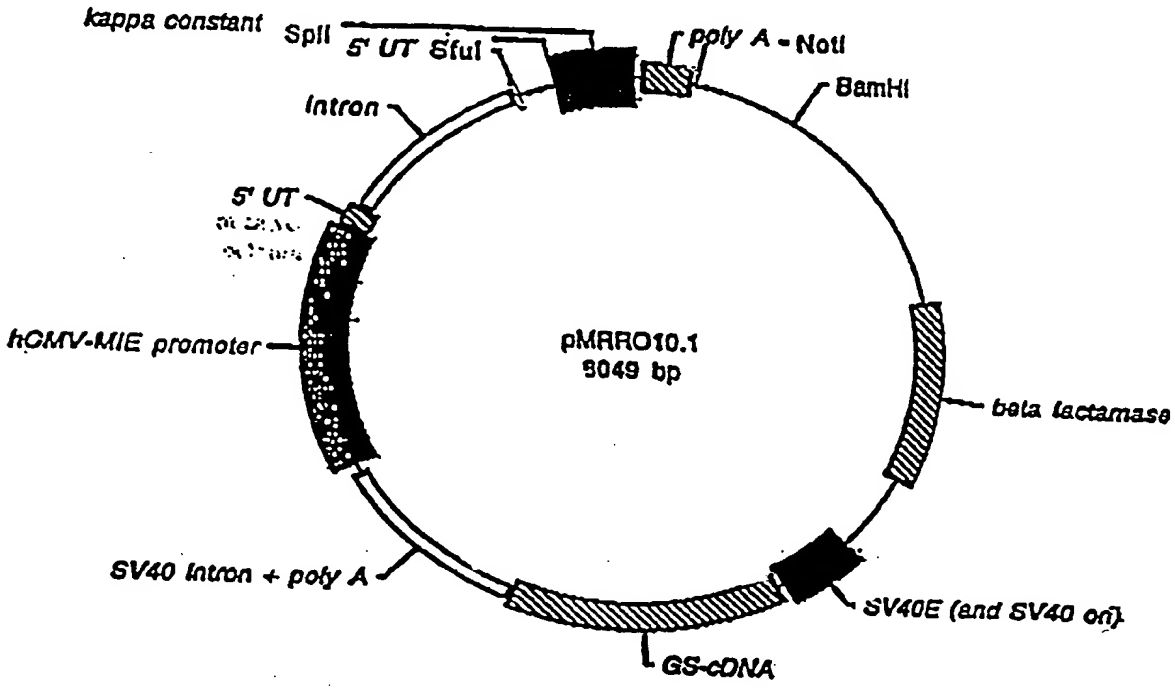

kappa constant
SpII
5' UT
SfuI
poly A - NotI
BamHI
Intron
5' UT
hCMV-MIE promoter
pMRRO10.1
9049 bp
beta lactamase
SV40 intron + poly A
SV40E (and SV40 ori)
GS-cDNA

FIG. 6A

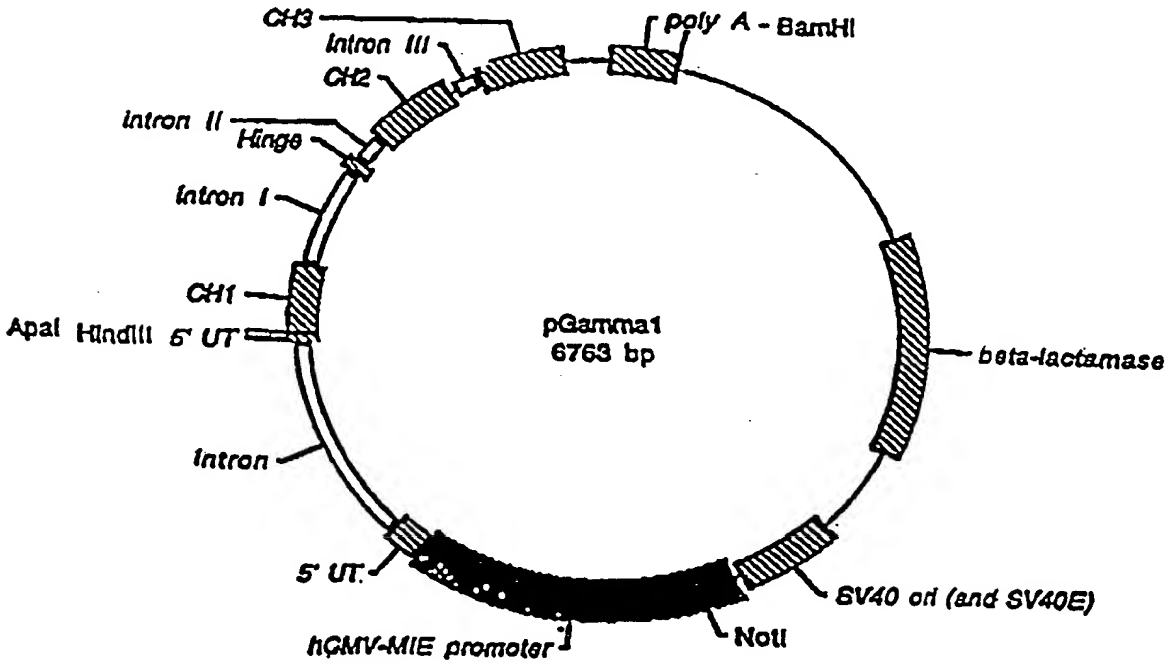

CH3
Intron III
CH2
Intron II
Hinge
Intron I
CH1
ApaI HindIII 5' UT
Intron
5' UT
hCMV-MIE promoter
NotI
SV40 ori (and SV40E)
beta-lactamase
poly A - BamHI
pGamma1
6763 bp

FIG. 6B

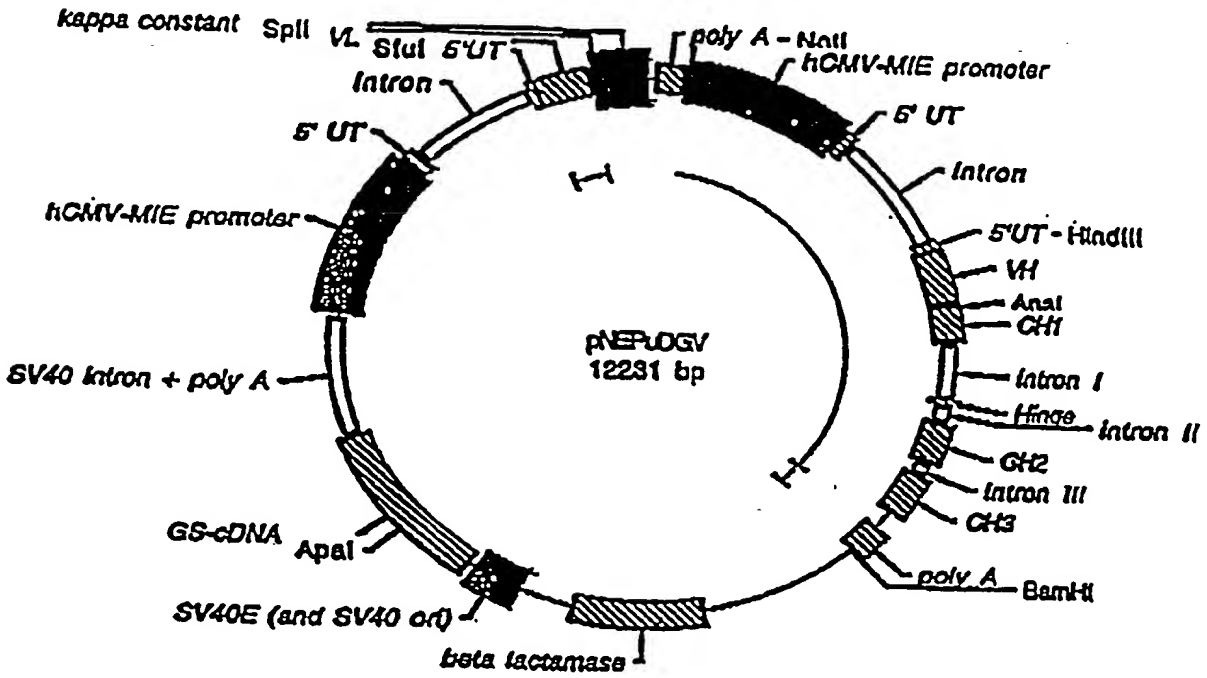

kappa constant
SpII
VL
StuI
5'UT
Intron
5' UT
hCMV-MIE promoter
SV40 intron + poly A
GS-cDNA
ApaI
SV40E (and SV40 ori)
beta lactamase
poly A - NotI
hCMV-MIE promoter
5' UT
Intron
5'UT - HindIII
VH
AnaI
CH1
Intron I
Hinge
Intron II
CH2
Intron III
CH3
poly A
BamHI
pNEPuDGV
12231 bp

FIG. 6C

ConVL1

EcoR1
GAA TTC

6

-19 (Leader) -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
63

V_L:

1 10 20
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
Thr
GAT ATC CAA ATG ACA CAA AGT CCT AGT AGT TTG AGT GCT AGT GTG GGA GAT CGG GTG
ACA 123

21 30 40
Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys
Pro
ATC ACA TGT CGG GCT AGT CAA AGT ATC AGT AAC TGT TTG GCT TGG TAT CAA CAA AAG
CCT 183

41 50 60
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro
Ser
GGA AAG GCT CCT AAG TTG TTG ATC TAT GCT GCT AGT AGT TTG GAG AGT GGA GTG CCT
AGT 243

61 70 80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Arg Phe Thr Leu Thr Ile Ser Ser Leu Gln
Pro
CGG TTC AGT GGA AGT GGA AGT GGA ACA CGG TTC ACC TTG ACC ATC AGT AGT TTG CAA
CCT 303

81 90 100
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Leu Pro Trp Thr Phe Gly
Gln
GAG GAT TTC GCT ACC TAT TAT TGT CAA CAA TAT AAC AGT TTG CCT TGG ACC TTC GGA
CAA 363

101
Gly Thr Lys Val Glu Ile Lys
GGA ACC AAG GTG GAG ATC AAG GAA TTC 390
Eco RI

FIG. 7A

ConVH1

EcoR1
GAA TTC 6

-19 (Leader) -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala
Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC
CAA AGT GCC CAA GCA 63

V_L:
1 10 20
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro
Gly Ala Ser Val Lys Val
CAG GTT CAG CTG GTG CAG TCT GGC GCT GAG GTG AAG AAG CCT
GGC GCT TCT GTG AAG GTG 123

21 30 35A 35B
40
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Ala Ile
Ser Trp Asn Trp Val Arg Gln Ala
TCT TGC AAG GCT TCT GGC TAC ACA TTC ACA TCT TAC GCT ATA
TCT TGG AAT TGG GTG AGG CAG GCT 189

41 50 60
Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Gly Asn
Gly Asp Thr Asn Tyr Ala
CCT GGC CAG GGC CTG GAG TGG ATG GGC TGG ATA AAT GGA AAT
GGA GAT ACA AAT TAC GCC 249

61 70 80
Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser
Thr Ser Thr Ala Tyr Met
CAG AAG TTC CAG GGA AGG GTG ACT ATA ACT GCT GAT ACT TCT
ACT TCT ACT GCT TAC ATG 309

81 82A 82B 82C 90
100
Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr
Cys Ala Arg Ala Pro Gly Tyr Gly Ser
GAG CTG TCT TCT CTG AGG TCT GAG GAT ACT GCT GTT TAC TAC
TGC GCT AGG GCT CCT GGC TAC GGC TCT 378

101 110
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
GAT TAT TGG GGA CAG GGA ACA CTG GTT ACA GTT TCT TCT GAA TTC
423

FIG. 7B

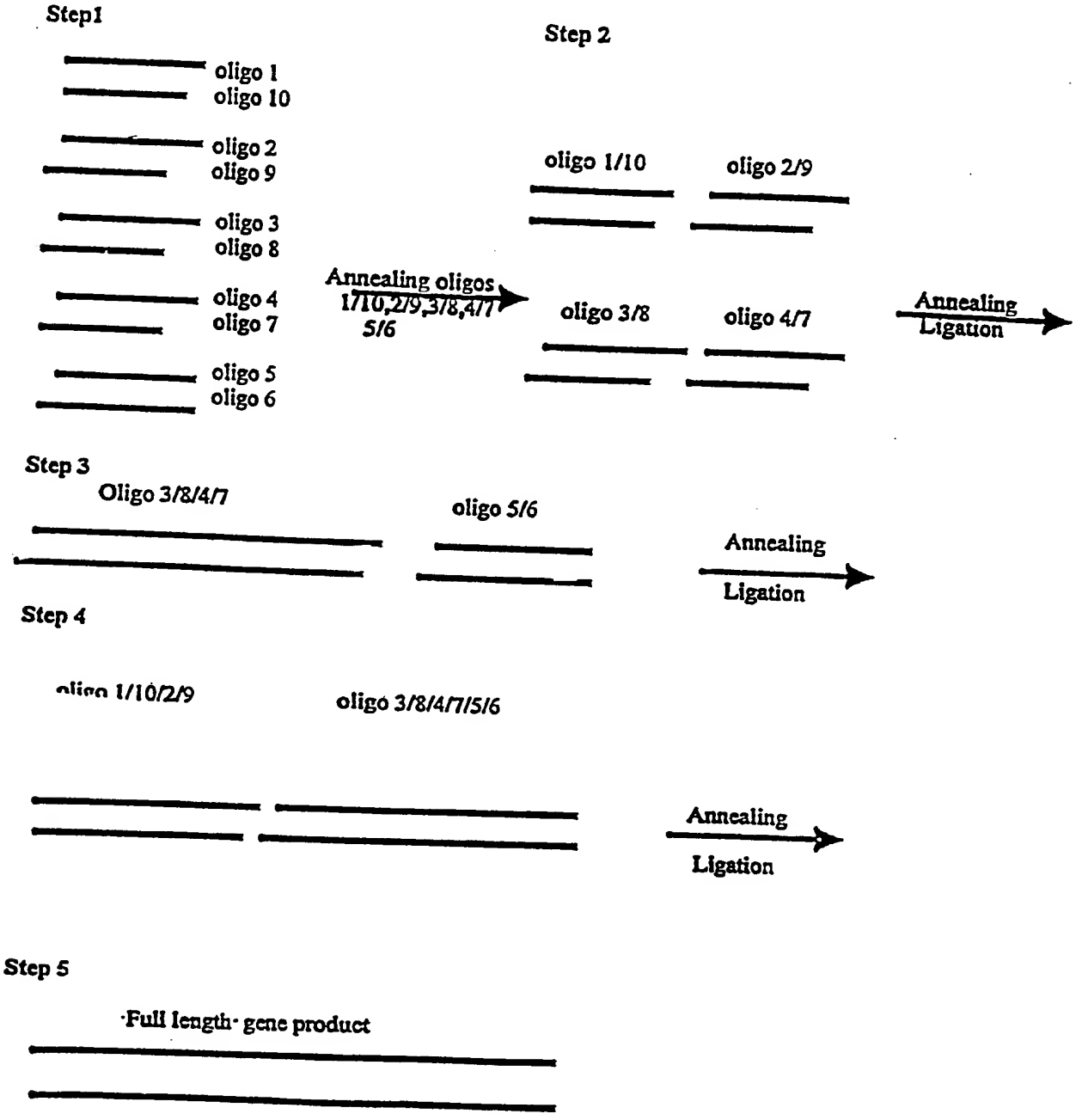

Step1
oligo 1
oligo 10
oligo 2
oligo 9
oligo 3
oligo 8
oligo 4
oligo 7
oligo 5
oligo 6
Annealing oligos 1/10,2/9,3/8,4/7 5/6
Step 2
oligo 1/10
oligo 2/9
oligo 3/8
oligo 4/7
Annealing Ligation
Step 3
Oligo 3/8/4/7
oligo 5/6
Annealing Ligation
Step 4
oligo 1/10/2/9
oligo 3/8/4/7/5/6
Annealing Ligation
Step 5
·Full length· gene product

FIG. 8

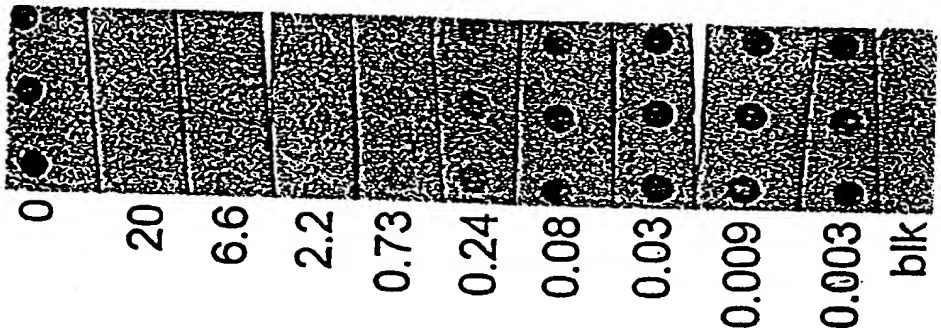
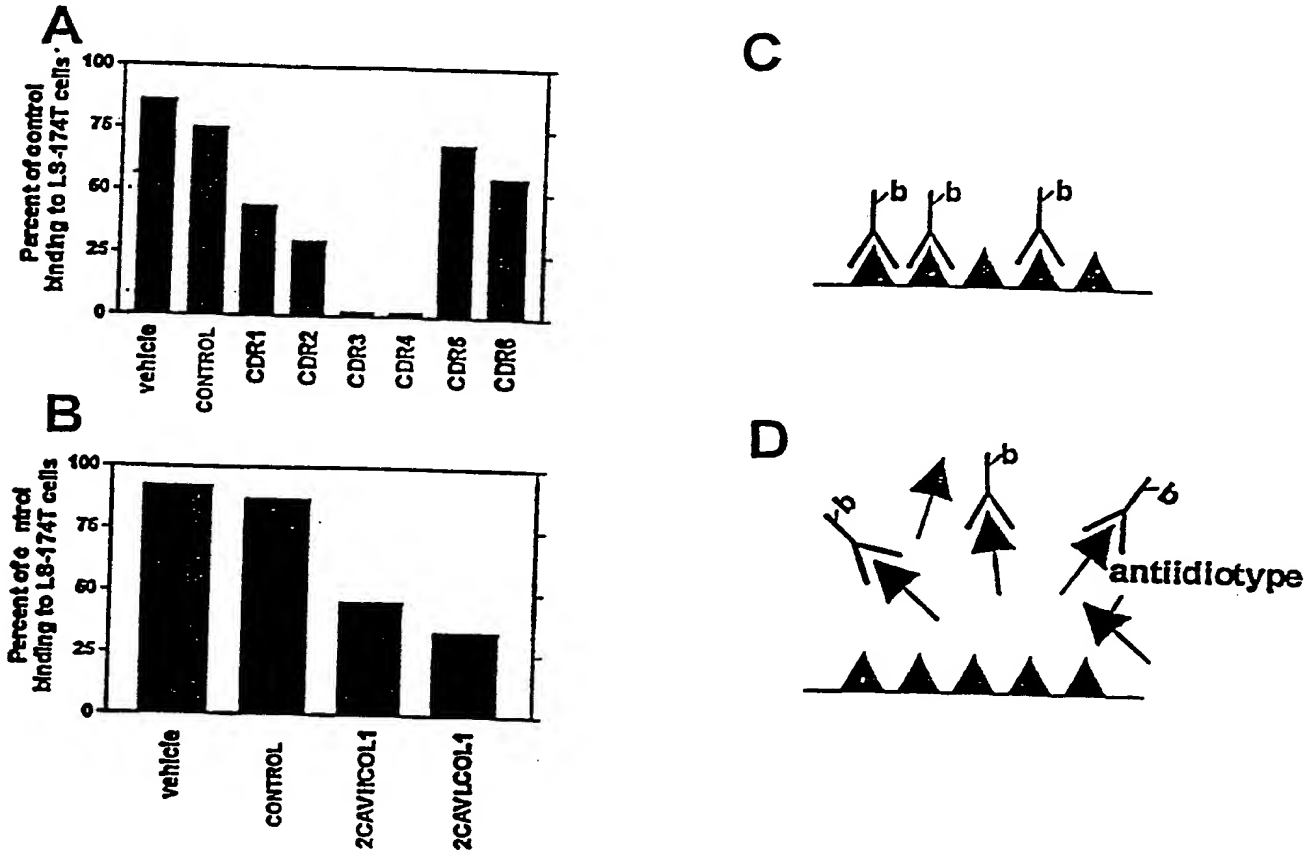


FIG. 9


A
Percent of control binding to L8-174T cells
100
75
50
25
0
vehicle
CONTROL
CDR1
CDR2
CDR3
CDR4
CDR5
CDR6
B
Percent of control binding to L8-174T cells
100
75
50
25
0
vehicle
CONTROL
2CAVHCOL1
2CAVLCOL1
C
b
b
b
D
b
b
b
antiidiotype

FIGS. 10A-D

cstacy
DSABL-1 GACATTGTGATGTCACAGTCTCCATCCTCCCTAGCTGTGTCAGTTGGAGAGAAGGTTACTATG 0.05
PAGE 63
DSABL-1c
GCAGCTCATAGTAACCTTCTCTCCAACTGACACAGCTAGGGAGGATGGAGACTGTGACATCACAATGTCTGC
TTGGGC 0.05 PAGE 78
MSAL-CDR1-1 ← GCT in MSALVAC-CDR1-1
AGCTGCGTCGGCAGCCTCCGAAGCAGCCCGCTCCAGAGCCCGCTGCTCCGATGGTACCAGCAGAAACCAG
GGCAGTCTCCTAAA 0.05 PAGE 84
MSAL-CDR1-1c
CTGCCCTGGTTTCTGCTGGTACCATCGGAGCAGCGGGCTCTGGAGCGGGCTGCTTCGGAGGCTGCCGAC
0.05 PAGE 69

HMVL1 GACATTGTGATGTCACAGTCTCCATCCTCCCTAGCTGTGTCAGTTGGAGAGAAGGTTACTATGAGCCCTAAGTCCAGT
HMVL2 CAGAGCCTTTTATATAGTAGCAATCAAAAGATCTACTTGGCCTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAA
HMVL3 CTGCTGATTTACTGGGCATCCACTAGGGAATCTGGGGTCCCTGATCGCTTCACAGGCGGGTGGATCTGGG
HMVL5 GCACAGCAATATTATAGATATCCTCGGACGTTCGGTGGAGGCACCAAGCTGGAAATCAACCGGGAATTC
HMVL8 ACGGCCTGTGAAGCGATCAGGGATCCCAGATTCCCTAGTGGATGCCCAGTAAATCAGCAGTTTAGGAGA
HMVL9 CTGCCCTGGTTCTCGCTGGTACCAGCCCAAGTAGATCTTTTGATTGCTACTATATAAAAGGCTCTGACTGGAGTT
HMVL10 AGCCCTCATAGTAACCTTCTCTCCAACTGACACAGCTAGGGAGGATGGAGACTGTGACATCACAATGTCTGCTTGGGC
HMVL6 GAATTCCCGTTTGATTTCCAGCTTGGTGCCTCCACCGAACGTCCCAGGATATCTATAATATTGCTGTGCGTAATAAAC

HMVL4
AAG AGA TTT GAG TCT CAG CAT CAG GAG TGT GAA GGC TGA AGA CGT GGG
AGT TTA TTA C

HMVL7
TG GGA GGT CTT CAG GCT TCA CAG TGC TGA TGG TGA GAG TGA AAT CTG
TCC CAG ATC C

FIG. 11

A

MSA-63 epitope DNA
GTC GGC AGC CTC CGA AGC AGC CCG CTC CAG AGC CCG CTG CTC CGA CCG CTC GTC
CAG AGC AGC CTC TGC TTG CTG TTC CTC TTG CTG CGA TAC AGC TGC GGC GAC GGC AGC
TGC AGC CGA CGA TAC TGC GAC TTG ACG GTG TGC ACG CGA ATG TAC TTG CTG CTG
CGA TTC ACG GAC GCG CCG CTC CCG CAG ACG TGC TGC GTC TTG AGC

B

MSA-63 protein sequence (Start residue 143 end residue 233)
Gln Pro Ser Glu Ala Ser Ser Gly Glu Val Ser Gly Asp Glu Ala Gly Glu Gln Val Ser Ser Glu Thr Asn Asp
Lys Glu Asn Asp Ala Met Ser Thr Pro Leu Pro Ser Thr Ser Ala Ala Ile Thr Leu Asn Cys His Thr Cys Ala
Tyr Met Asn Asp Asp Ala Lys Cys Leu Arg Gly Glu Gly Val Cys Thr Thr Gln Asn Ser

C

MSA-63 oligo

MSA1
GTC GGC AGC CTC CGA AGC AGC CCG CTC CAG AGC CCG CTG CTC CGA

MSA2
AGC CCG CTG CTC CGA CCG CTC GTC CAG AGC AGC CTC TGC TTG CTG

MSA3
AGC CTC TGC TTG CTG TTC CTC TTG CTG CGA TAC AGC TGC GGC GAC

MSA4
TAC AGC TGC GGC GAC GGC AGC TGC AGC CGA CGA TAC TGC GAC TTG

MSA5
CGA TAC TGC GAC TTG ACG GTG TGC ACG CGA ATG TAC TTG CTG CTG

MSA6
ATG TAC TTG CTG CTG CGA TTC ACG GAC GCG CCG CTC CCG CAG ACG

MSA7
CGA TTC ACG GAC GCG CCG CTC CCG CAG ACG TGC TGC GTC TTG AGC

FIGS. 12A-C

A

SP-10 Epitope
GAA TTC CAG CCT TCA GGT GAA CAT GGC TCC GGT GAA CAG CCT TCT GGT GAG CAG
GCC TCG GGT GAA CAG CCT TCA GGT GAG CAC GCT TCA GGG GAA CAG GCT TCA GGT
GCA CCA ATT TCA AGC ACA TCT ACA GGC ACA ATA TTA AAT TGC TAC ACA TGT GCT TAT
ATG AAT GAT CAA GGA AAA TGT CTT CGT GGA GAG GGA ACC TGC ATC ACT CAG AAT TC

B

SP-10 protein sequence
Gln Pro Ser Gly Glu His Gly Glu Gln Pro Ser Gly Glu Gln Ala Ser Gly Glu Gln Pro Ser gly Glu His Ala
Ser Gly Glu Gln Ala Ser Gly Ala Gln Ile Ser Ser Thr Ser Thr Gly Thr Ile Leu Asn Cys Tyr Thr Cys Ala
Tyr Met Asn Asp Gln Gly Lys Cys Leu Arg Gly Glu Gly Thr Cys Ile Thr Gln Asn

C

Oligo SP1:
GAA TTC CAG CCT TCA GGT GAA CAT GGC TCC GGT GAA CAG CCT TCT GGT GAG CAG
GCC TCG GGT GAA CAG CCT TAG

Oligo SP2:
GTG AGC ACG CTT CAG GGG AAC AGG CTT CAG GTG CAC CAA TTT CAA GCA CAT CTA
CAG GCA CAA TAT TAA ATT GCT

Oligo SP3:
ACA CAT GTG CTT ATA TGA ATG ATC AAG GAA AAT GTC TTC GTG GAG AGG GAA CCT
GCA TCA CTC AGA ATT C

Oligo SP3a(3Cys-> Ala):
ACA CAG CAG CTT ATA TGA ATG ATC AAG GAA AAG CAC TTC GTG GAG AGG GAA
CCG CAA TCA CTC AGA ATT C

Oligo SP4:
GAA TTC TGA GTG ATG CAG GTT CCC TCT CCA CGA AGA CAT TTT CCT TGA TCA TTC ATA
TAA GCA CAT GTG TAG CAA TTT A

Oligo SP4a (3Cys->Ala):
GAA TTC TGA GTG ATT GCG GTT CCC TCT CCA CGA AGT GCT TTT CCT TGA TCA TTC ATA
TAA GCT GCT GTG TAG CAA TTT A

Oligo SP5:
ATA TTG TGC CTG TAG ATG TGC TTG AAA TTG GTG CAC CTG AAG CCT GTT CCC CTG AAG
CGT GCT CAC CTG AAG GCT

Oligo SP6:
GTT CTC CCG AGG CCT GCT CAC CAG AAG GCT GTT CAC CGG AGC CAT GTT CAC CTG
AAG GCT GGA ATT C

FIGS. 13A-C

LDH-C_4 Epitope:

Oligo LDH1:
TCG TGC CAG TTC CTC GTC GAC TAG CTC TTC GAC TAG CTC CTG CTG CTC TTG TCG GTC
ACG GAA TTC

Oligo LDH2:
GAA TTC CGT GAC CGA CAA GAG CAG CAG GAG CTA GTC GAA GAG CTA GTC GAC GAG
GAA CTG GCA CGA CGG GTT CGT

FIG. 14

Leader:

```
-19                                                                  -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
```

V_L: Ala GCT in vaccine

```
1                                   10                                  20
Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala Val Ser Val Gly Glu Lys Val Thr
GAC ATT GTG ATG TCA CAG TCT CCA TCC TCC CTA GCT GTG TCA GTT GGA GAG AAG GTT ACT

21                                27 A  B  C  D  E  F          30
Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser Ser Asn Gln Lys Ile Tyr Leu Ala Trp Tyr Gln Gln Lys Pro
ATG AGC TGC AAG TCC AGT CAG AGC CTT TTA TAT AGT AGC AAT CAA AAG ATC TAC TTG GCC TGG TAC CAG CAG AAA CCA
                                    CDR1

41                                  50                                  60
Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Asp
GGG CAG TCT CCT AAA CTG CTG ATT TAC TGG GCA TCC ACT AGG GAA TCT GGG GTC CCT GAT
                                    CDR2

61                                  70                                  80
Arg Phe Thr Gly Gly Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Lys Ala
CGC TTC ACA GGC GGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGT GTG AAG GCT

81                                  90                                  100
Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr Arg Tyr Pro Arg Thr Phe Gly Gly
GAA GAC CTG GCA GTT TAT TAC TGT CAG CAA TAT TAT AGA TAT CCT CGG ACG TTC GGT GGA
                                    CDR3

101
Gly Thr Lys Leu Glu Ile Lys Arg
GGC ACC AAG CTG GAA ATC AAA CGG
```

FIG. 15

2CAVHCOL1

VHC1 5'GAATTCATGGCTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGCCCAAAGTGCCC
AAGCACAGATCCAGTTGGTGCA 3'

VHC2 5'GTCTGGACCTGAGCTGAAGAAGCCTGGAGAGACAGTCAAGATCTCCGCTAAGGCTTC
TGGGTATACCTTCACAAACTAG 3'

VHC3 5'GAATGAACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGGGCTGGAT
AAACACCTACACTGGAGAGCCAACA 3'

VHC4 5'TATGCTGATGACTTCAAGGGACGGTTTGCCTTCTCTTTGGAAACCTCTGCCAGCACT
GCCTATTTGCAGATCAACACCT 3'

VHC5 5'CAAAAATGAGGACACGGCTACATATTTCGCTGCAAGAGCCTACTATGGTAAATAC
TTTGACTACGAATTC 3'

VHC6 5'GAATTCGTAGTCAAAGTATTTACCATAGTAGGCTCTTGCAGCAAATATG 3'

VHC7 5'TAGCCGTGTCCTCATTTTTGAGGTTGTTGATCTGCAAATAGGCAGTGCTGGCAGA
GGTTTCCAAAGAGAAGGCAAACCGT 3'

VHC8 5'CCCTTGAAGTCATCAGCATATGTTGGCTCTCCAGTGTAGGTGTTTATCCAGCCCAT
CCACTTTAAACCCTTTCCTGGAGC 3,

VHC9 5'CTGCTTCACCCAGTTCATTCCATAGTTTGTGAAGGTATACCCAGAAGCCTTAGCGG
AGATCTTGACTGTCTCTCCAGGCT 3'

VHC10 5'TCTTCAGCTCAGGTCCAGACTGCACCAACTGGATCTGTGCTTGGGCACTTTG GGC
AGCTGCCATCAGGAATAGCAAGGTCCACACCCAAGCCATGAATTC 3'

FIG. 16A

2CAVLCOL1

VLC1 5'AGTATTGTGATGACCCAGACTCCCAAATTCCTGCTTGTATCAGCAGGAGACAGGGTT
ACCATA 3'

VLC2 5'ACCTGCAAGGCCAGTCAGAGTGTGAGTAATGATGTAGCTTGGTACCAACAGAAAACC
AGGGCAG 3'

VLC3 5'TCTCCTAAACTGCTGATATACTATGCATCCAATCGCTACACTGGAGTCCCTGATCGCT
TCACTGGCAGT 3'

VLC4 5'GGATATGGGACGGATTTCACTTTCACCATCAGCACTGTGCAGGCTGAAGACCTGGCA
GTTTAT 3'

VLC5 5'TTCTGYCAGCAGGATTATAGCTCTCCGCTCACGTTCGGTGCTGGGACCAAGCTGGAG
CTGAAAGAATTC 3'

VLC6 5'GAATTCTTTCAGCTCCAGCTTGGTCCCAGCACCGAACGTGAGCGGAGAGCTATAATC
CTGCTGACAGAAATAAACTGC 3'

VLC7 5'CAGGTCTTCAGCCTGCACAGTGCTGATGGTGAAAGTGAAATCCGTCCCATATCCA
CTGCCAGT 3'

VLC8 5'GAAGCGATCAGGGACTCCAGTGTAGCGATTGGATGCATAGTATATCAGCAGCTTAG
GAGACTGCCCTGG 3'

VLC9 5'TTTCTGTTGGTACCAAGCTACATCATTACTCACACTCTGACTGGCCTTGCAGGTTA
TGGTAAC 3'

VLC10 5'CCTGTCTCCTGCTGATACAAGCAGGAATTTGGGAGTCTGGGTCATCACAATACTT
GCTTGGGC 3'

VLC11 5'TTCGCTCAGCAGGATTATAGCTCTCCGCTCACGTTCGGTGCTGGGACCAAGCTGG
AGCTGAAAGAATC 3'

VLC12 5'GAATTCTTTCAGCTCCAGCTTGGTCCCAGCACCGAACGTGAGCGGAGAGCTATAA
TCCTGCTGAGCGAAATAAACTGC 3'

FIG. 16B

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 16/00
US CL : 530/387.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/131.1, 133.1, 134.1; 530/350, 387.1, 387.2, 388.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Medline, West

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,637,300 (DUNBAR et al.) 10 June 1997 (10.06.1997), column 10-11	1,4,5,10,21,22,25,26, 31 --- 11,14,15,20
Y	SEFERIAN et al. Antibody synthesis induced by endogenous internal immages. Applied Biochemisrty and Biotechnology 1994, Vol. 47, see pages 213-227.	1, 7-11, 17-21, 28-31
Y	CARRON et al. Characterization of antibodies to idiotypic determinants of monoclonal anti-sperm antibodies. Biology of Reproduction 1988, Vol. 38, see abstract.	1,2,5,10-12,15, 20-23, 26,31
Y	TRIPATHI et al. Antigen mimicry by an anti-idiotypic antibody single chain variable fragment. Molecular Immunology 1998, Vol. 35, see pages 853-863.	1,7,8-11,17-22,28-31
Y	US 5,208,146A (IRIE) 04 May 1993 (04.05.1993) see column 1 and 5.	1,7-11, 17-21, 28-31
Y	US 5,436,157A (HERR et al.) 25 July 1995 (25.07.1995) see columns 1-3.	2,3,6,12,13,16,23,24, 27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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(54) Title: SELECTIVE ALTERATION OF ANTIBODY IMMUNOGENICITY

(57) Abstract

The present invention relates to a simple process for the modification of, e.g., anti-TAA antibodies, which alters their immunogenicity so that their ability to induce an anti-isotypic response is selectively diminished, while they remain able to elecit an anti-idiotypic response. The latter is of potential immunotherapeutic value, i.e., by activation of the idiotype-anti-idiotype network. This modification takes the form of a controlled and partial reduction of the antibody; effector regions are retained. The invention should permit repeat injections (for diagnosis and therapy) and reduce HAMA interference in serodiagnostic assays.

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SELECTIVE ALTERATION OF ANTIBODY IMMUNOGENICITY

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to a method of altering the immunogenicity of antibodies so that, upon administration to a suitable subject, an immune response is elicited which is predominantly anti-idiotypic rather than anti-isotypic in character.

Description of the Background Art

All vertebrates possess a surveillance mechanism, called the immune system, that protects them from pathogenic microorganisms (including viruses), multicellular parasites, and cancer cells. The immune system specifically recognizes and selectively eliminates these undesirables by a process known as the immune response. One of its two important subsystems is the humoral immune system, which relies on antibodies, produced in quantity by plasma cells, that circulate through the blood and the lymphatic fluid.

The first step in the immune response is the recognition of the presence of a foreign entity. Antigens are molecules which are subject to immune recognition. The portion of an antigen to which an antibody binds is called its antigenic determinant, or epitope. Not all antigens are capable of eliciting a response, as opposed to simple molecular recognition, from the immune system. Antigens which can elicit an immune response are termed immunogens, and are usually macromolecules, such as proteins, nucleic acids, carbohydrates, and lipids, of at least 5000 Daltons molecular weight. However, many small nonimmunogenic molecules, termed haptens, can stimulate an immune response if associated with a large carrier molecule.

Antibodies, also known as immunoglobulins, are proteins. They have two principal functions. The first is to recognize (bind) foreign antigens. The second is to mobilize other elements of the immune system to destroy the foreign entity.

The basic unit of immunoglobulin structure is a complex of four polypeptides -- two identical low molecular weight ("light") chains and two identical high molecular weight ("heavy") chains,

linked together by both nocovalent associations and by disulfide bonds. Different antibodies will have anywhere from one to five of these basic units. The immunoglobulin unit may be represented schematically as a "Y". Each branch of the "Y" is formed by the amino terminal portion of a heavy chain and an associated light chain. The base of the "Y" is formed by the carboxy terminal portions of the two heavy chains. The node of the "Y" is the so-called hinge region, and is quite flexible. Five human antibody classes (IgG, IgA, IgM, IgD and IgE), and within these classes, various subclasses, are recognized on the basis of structural differences, such as the number of immunoglobulin units in a single antibody molecule, the disulfide bridge structure of the individual units, and differences in chain length and sequence. The class and subclass of an antibody is its isotype.

The amino terminal regions of the heavy and light chains are far more diverse in sequence than the carboxy terminal regions, and hence are termed the variable domains. This is the part of the antibody whose structure confers the antigen-binding specificity of the antibody. A heavy variable domain and a light variable domain together form a single antigen-binding site, thus, the basic immunoglobulin unit has two antigen-binding sites. The walls of the antigen-binding site are defined by hypervariable segments of the heavy and light variable domains. Binding site diversity is generated both by sequence variation in the hypervariable region and by random combinatorial association of a heavy chain with a light chain. Collectively, the hypervariable segments are termed the paratope of the antibody; this paratope is essentially complementary to the epitope of the cognate antigen.

The carboxy terminal portion of the heavy and light chains form the constant domains. While there is much less diversity in these domains, there are, first of all, differences from one animal species to another, and secondly, within the same individual, there will be several different isotypes of antibody, each having a different function.

The IgG molecule may be divided into homology units. The light chain has two such units, the V_L and C_L, , and the heavy chain has four, designated V_H, C_H1, C_H2 and C_H3. All are about

110 amino acids in length and have a centrally located intrachain disulfide bridge that spans about 60 amino acid residues. The sequences of the two V-region homology units are similar, as are the sequences of the four C-region homology units. These homology units in turn form domains. The two variable domains have already been mentioned; there are also four constant domains. Mild proteolytic digestion of IgG results in the production of certain fragments of interest. V-C1 is Fab; C_H2-C_H3 is Fc; $(V\text{-}C1)_2$ is $(Fab')_2$, V-C1-C2 is Fabc, and V alone is Fv.

While the variable domains are responsible for antigen binding, the constant domains are charged with the various effector functions: stimulation of B cells to undergo proliferation and differentiation, activation of the complement cell lysis system, opsonization, attraction of macrophages to ingest the invader, etc. Antibodies of different isotypes have different constant domains and therefore have different effector functions. The best studied isotypes are IgG and IgM.

If a specific antibody from one animal is injected as an immunogen into a suitable second animal, the injected antibody will elicit an immune response. Some of these anti-antibodies will be specific for the unique epitopes (idiotopes) of the variable domains of the injected antibodies; these epitopes are known collectively as the idiotype of the primary antibody and the secondary (anti-) antibodies which bind to these epitopes are known as anti-idiotypic antibodies. Other secondary antibodies will be specific for the epitopes of the constant domains of the injected antibodies and hence are known as anti-isotypic antibodies. (The term "anti-isotypic" antibodies, as used herein, includes antibodies that are merely species-specific as well as antibodies which are also class or subclass-specific.)

The "network" theory states that antibodies produced initially during an immune response will carry unique new epitopes to which the organism is not tolerant, and therefore will elicit production of secondary antibodies (Ab2) directed against the idiotypes of the primary antibodies (Ab1). These secondary antibodies likewise will have an idiotype, which will induce production of tertiary antibodies (Ab3), and so forth.

It also suggests that some of those secondary antibodies will have a binding site which is the complement of the complement of the original antigen, and thus will reproduce the "internal image" of the original antigen. In other words, an anti-idiotypic antibody may be a surrogate antigen.

There are four major types of anti-idiotypic antibodies. The alpha-type is one which binds an epitope remote from the paratope of the primary antibody. The beta-type is one whose paratope mimicks the epitope of the original antigen. The gamma-type binds near enough to the paratope of the primary antibody to interfere with antigen binding. The epsilon type recognizes an idiotypic determinant that mimicks a constant domain antigenic structure. Moreover, anti-isotypic antibodies may be heavy chain-specific or light chain-specific.

"Active immunotherapy" is the administration of an antigen, in the form of a vaccine, to a patient, so as to elicit a protective immune response. "Passive immunotherapy" involves the administration of antibodies to a patient. Antibody therapy is conventionally characterized as passive since the patient is not the source of the antibodies. However, the term passive is misleading because the patient can produce anti-idiotypic secondary antibodies which in turn provoke an immune response which is cross-reactive with the original antigen.

As stated by Koprowski (3), a traditional approach to cancer immunotherapy is to administer anti-tumor antibodies, i.e., antibodies which recognize an epitope on a tumor cell, to patients. However, the development of the "network" theory led her and others (4) to suggest the direct administration of exogenously produced anti-idiotype antibodies, that is antibodies raised against the idiotype of an anti-tumor antibody. Koprowski assumes that the patient's body will produce anti-antibodies which will not only recognize these anti-idiotype antibodies, but also the original tumor epitope.

Koprowski's exogenous anti-idiotypic antibodies are the product of a rather complex production process. Polyclonal anti-idiotypic antibodies must be separated from other antibodies in the serum of the animal. The use of monoclonal anti-idiotypic antibodies simplifies purification to some degree, but at the

cost of a laborious screening procedure to identify hybridomas secreting the desired anti-idiotypic antibody. Then these cells must be expanded in culture. Finally, once a production culture is developed, the antibodies still must be recovered, purified and tested. Applicants believe it to be preferable to stimulate in vivo production of the anti-idiotypic antibody.

It is of course true that Applicants' antibodies must also be purified. However, Applicants need only distinguish between antibodies which bind to the immunogen and those which do not. The proponents of exogenous anti-idiotypic antibody therapy must differentiate antibodies which bind to the same immunogen, but in different places.

In a related vein, it has been suggested that one may administer a synthetic polypeptide that substantially immunologically corresponds to an idiotypic epitope of an antibody directed against an antigen of interest (5). However, this polypeptide must be synthesized and purified. Moreover, this methodology requires knowledge of the sequence of the antigen binding site of the anti-idiotypic antibody.

Sources of human antibodies are limited to subjects already suffering from the disease of interest, as it is unethical to introduce a disease into a subject merely so the subject will begin producing antibodies which may be harvested. Because of the difficulties of collecting human antibodies, clinicians rely on antibodies of nonhuman origin, such as mouse antibodies. Unfortunately, besides eliciting an anti-idiotypic response, these mouse antibodies, when administered to humans, also provoke production of secondary human anti-mouse antibodies (HAMA) directed against mouse-specific and mouse isotype-specific portions of the primary antibody molecule. This immune reaction occurs because of differences in the primary amino acid sequences in the constant regions of the immunoglobulins of mice and humans. Both IgG and IgM subclasses of HAMA have been detected. The IgG response appears later, is longer-lived than the typical IgM response, and is more resistant to removal by plasmapheresis.

Clinically, the development of HAMA increases the likelihood of anaphylactic or serum sickness-like reactions to subsequent

administration of murine immunoglobulins. These secondary antibodies reduce the efficacy of repeat immunotherapy by complexing subsequently administered mouse antibody (31). HAMA-induced increases in the clearance of the injected antibody or fragment can result in reduced tumor localization, enhanced uptake into liver and spleen, and tumor escape from therapy. HAMA can also cause interference with immunodiagnosis, and thereby hinder monitoring of the progress of the disease and the effectiveness of the course of treatment.

The anti-isotype response has been avoided in prior immunoimaging work through the use of monovalent Fab fragments or divalent $(Fab')_2$ fragments. These fragments lack most of the constant region and therefore present only a very limited opportunity for anti-isotype binding (1). Moreover, they lack the effector functions of a more intact antibody and therefore will not activate complement, or bind to an Fc receptor on a killer cell. Accordingly, such fragments, which lack most or all of the constant region, are not normally used in immunotherapy.

Another approach is to conjugate a tolerogen, such as polyethylene glycol, to the antibody to reduce its immunogenicity (2). Unfortunately, PEGging an antibody also diminishes its ability to elicit an anti-idiotypic response.

Wagner, et al. (6) radioimmunoimaged 12 patients with ovarian carcinomas using Iodine-131 labeled $F(ab')_2$ fragments of the anti-CA125 mouse antibody OC125. All patients had been treated in the same manner by surgery followed by chemotherapy. Five of the patients developed anti-idiotypic antibodies against the imaging antibody. In 1989, only these five patients were still alive. Wagner, et al. suggested that their longterm survival was attributable to their development of anti-idiotypic antibodies against the OC125 fragments, and hence to induction of the idiotypic network. While Wagner et al.'s fragments may have exerted a serendipitous immunotherapeutic effect through generation of Ab3, they nonetheless lack the effector functions of conventional immunotherapeutic agents. Moreover, because these fragments are more rapidly cleared from the bloodstream, they are less useful than intact antibody for immunotherapy.

The use of intact antibody (Ab1) to activate the idiotype-anti-idiotype network, while potentially enhancing the immunotherapeutic utility of the antibody, would raise the issue of problems with anti-isotypic responses, as previously mentioned. Wagner et al. did not need to address the possibility of an anti-isotypic response since he had administered fragments lacking most of the constant region.

A methodology is urgently needed that allows use of animal antibodies in human therapy, with in vivo stimulation of an endogenous anti-idiotypic response and without concomitant stimulation of a substantial anti-isotypic response (the term here including a species-specific response), which does not require use of antibody fragments which lack constant regions.

All references, including patents and patent applications, which are cited anywhere in this specification are hereby incorporated by reference. No admission is made that any cited reference constitutes prior art, or pertinent prior art.

SUMMARY OF THE INVENTION

Applicants have discovered that the immunogenic character of antibodies may be modified so as to substantially eliminate the anti-isotype response while substantially preserving the anti-idiotype response to the antibodies.

If the anti-isotype response is eliminated, it may be possible to repeatedly administer an antibody to a patient without fear of putting the patient into anaphylactic shock brought on by an adverse immune reaction between the exogenous antibody and previously elicited anti-isotype anti-antibodies. Retention of the anti-idiotype response is advantageous, however, as the anti-idiotype anti-antibody mimics the original antigen, and thereby can elicit production in the patient of endogenous antibodies which likewise recognize the original antigen. Elimination of the anti-isotypic response will also facilitate subsequent immunosurveillance of the patient by in vitro and in vivo immunodiagnostic techniques, as interference from anti-isotypic anti-antibodies will be avoided.

While simply removing the Fc portion of an antibody is likely to substantially eliminate its ability to elicit an anti-isotype response, the use of antibody fragments such as Fab and Fab' fragments has other disadvantages. These fragments have a shorter residency time in the bloodstream, and therefore are less desirable from a therapeutic standpoint than a whole antibody. They also fail to provide all of the effector functions associated with intact antibody, which reduces their therapeutic effectiveness. Indeed, they may actually interfere with the action of endogenous antibodies, which have the effector function, by blocking the antigenic determinants. Thus, while they have some therapeutic value through eliciting production of Ab3, in general they are not suitable as immunotherapeutic agents.

Instead, applicants treat the antibody with a reagent that is capable of reducing certain of the disulfide (-S-S-) bridges of the immunoglobulin, thereby generating free sulfhydryl groups, but without fragmenting the antibody sufficiently to abolish effector function.

The reduction also results in a denaturation of the heavy chain conformation, and thereby substantially eliminates anti-heavy chain or isotype antibody response. It is also believed that under certain circumstances the anti-idiotypic response can be increased in both an absolute as well as a relative sense. While applicants do not wish to be bound to this theory, it is believed that the cleavage of certain disulfides results in greater conformational flexibility in the critical antigen binding variable and hypervariable regions, exposing areas which previously were subject to steric hindrance, and therefore to a greater propensity toward anti-idiotype responses. However, an absolute increase in the anti-idiotypic response is not required for the practice of this invention.

The present invention also relates to an improved method of reducing, and, if desired, radiolabeling antibodies. These antibodies may be used for radioimmunotherapy, or for radioimmunoimaging (with a reduced isotypic HAMA response to interfere with subsequent immunotherapy).

The appended claims are hereby incorporated by reference as a further recitation of the preferred embodiments.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention relates to the production of reduced antibodies and their use, alone or in combination with other agents, as immunotherapeutic agents.

All immunoglobulin G molecules consist of two heavy and two light polypeptide chains covalently bound to each other through several disulphide bridges between cysteine amino acids. In addition to these interchain bridges, there are a greater number of intrachain disulphide bonds which also aid in the maintenance of the tertiary structure of the molecule. Under reductive conditions, these bridges can be cleaved to the corresponding sulphydryl forms.

There are numerous techniques for preparing reduced antibodies. In general, the compounds used fall into three categories - the classical reducing agents comprising organic (for example, formamidine sulfonic acid) and inorganic (for example, mercurous ion, stannous ion, cyanide ion, sodium

cyanoborohydride, sodium borohydride, etc.) compounds, the thiol exchange reagents (for example, dithiothreitol, mercaptoethanol, mercaptoethanolamine) and protein reductants (for example, thioredoxin). Exposure of immunoglobulin-G molecules (or their fragments) to these compounds results in somewhat selective reduction of disulphides to form various sulphydryl groups. Under continuing reductive conditions, these sulphydryl groups remain, resulting in an at least partially disulphide reduced protein molecule, and at least potentially changing the tertiary structure of the immunoglobulin. The effect of the reduction on the conformation and immunoreactivity of the antibody molecule is dependent on the degree of reduction.

The reduction results in a denaturation of the heavy chain conformation, and thereby substantially reduces or even eliminates anti-heavy chain or isotype antibody response.

While totally reduced antibody molecules are potentially usable, it is likely that their affinity for antigen will be substantially diminished. Consequently, it is preferable to control the degree of reduction of the antibody so that it retains at least some of its intra- and/or inter-chain disulphide bonds. The most susceptible disulphide bridges are those in the hinge region and therefore under appropriate conditions these can be preferentially cleaved. This potentially allows greater movement of the critical antigen binding variable and hypervariable regions and may expose previously hindered areas of these regions. With some antibodies, this may lead to an enhancement of the anti-idiotype human anti-mouse antibody response.

Reducing agents potentially useful for the selective elimination of the isotype immunogenicity of the antibody are readily tested for suitability by the HAMA assay described in this specification, or by other assays capable of differentiating anti-idiotypic and anti-isotypic HAMA (31).

The HAMA assay described in the Examples is a two-step indeirect radioimmunoassay. Beads which have been precoated with goat anti-mouse antibody are incubated with a second murine antibody or fragment to form the complex that captures HAMA. In order to measure a generalized HAMA response, only a nonspecific

antibody, e.g. an irrelevant murine IgG monoclonal antibody, is used as the second antibody. In order to measure an anti-idiotypic HAMA response, the particular antibody administered to the patients is used on some beads and the nonspecific control antibody is used on others.

After the incubation with the second murine antibody or fragment, the beads are washed to remove any unbound antibodies. The beads are now considered "primed" to capture HAMA. After washing, diluted test serum is added and incubated with the primed beads. HAMA present in the serum is captured or linked to the primed beads during this incubation. Following a second wash, the beads are incubated with a radiolabeled tracer antibody, e.g., Iodine-125 labeled polyclonal anti-human antibodies, which binds to captured HAMA. Any unbound radiolabeled antibody is removed by a final wash before measuring the amount of bound radioactivity.

Results obtained using the positive (anti-mouse Ig serum) and negative (human serum) controls supplied in the kit are used to calculate the HAMA limit.

About 9% of a normal population has been found to exhibit positive HAMA responses before in vivo administration of murine immunoglobulin. Certain patient groups have higher preinjection HAMA responses, so it is desirable to obtain a pre-injection baseline sample.

The present invention is not limited to any particular method of determining anti-isotypic and anti-idiotypic HAMA, or any particular reagents for use therein. It is believed that the Behringerwerke ENZYGNOST HAMA micro assay has the components needful for measuring both HAMA responses, though the kit does not explain how to perform this calculation. Measurement of anti-idiotypic response is reported in, e.g., Reinsberg, et al., Clin. Chem. ,36: 164-167 (1990); GOldman-Leikin, et al., Exp. Hematol. 16: 861-864 (1988).

While we have spoken in terms of the HAMA response, we could as well have addressed any immune response of one animal to antibodies derived from a different species of animal.

The reduced antibody elicits at least some anti-idiotypic anti-antibody response but no more than a substantially

decreased, if any, anti-isotype response, relative to the unreduced antibody. Desirably, no more than 20%, and more desirably, no more than 5%, of the anti-isotypic response of the subject to the antibody is retained after reduction. Most desirably, the anti-isotypic response is essentially eliminated. Preferably, at least 25%, more preferably at least 50%, still more preferably at least 80%, and most preferably, at least 95%, of the anti-idiotypic response of the subject to the antibody is left under these circumstances. Preferably, the reduction in the anti-isotypic response is substantially greater than the reduction in the anti-idiotypic response.

While it is preferable that the reduced antibodies of the present invention retain their Fc and hinge regions, it is also possible to reduce antibody fragments that possess only a portion of the normal Fc region or hinge region, such as $(Fab')_2$.

If desired, the reduced antibody may be radiolabeled with pertechnetate or perrhenate to produce a radiolabeled antibody which may be used for radioimmunoimaging as well as radioimmunotherapy. The radioisotope may be one with a cytotoxic effect and therefore of therapeutic value if the antibody is directed against an antigen of an undesirable cell, such as a cancer cell.

A particularly preferred reduction method employs $SnCl_2$ as the reducing agent. Preferably, the molar ratio of this reducing agent to the antibody is in the range of 20:1 to 100:1; the most preferred value is about 40:1. Use of a high level of stannous ion increases the chance of damaging or fragmenting the antibody and also increases the likelihood of Tc-99m-Sn(II) formation competing significantly with the MAb-Tc-99m reaction.
The concentration of the antibody may be in the range of 1 to 10 mg/mL; preferably 5mg/mL.

The reaction buffer preferably is a tartrate (e.g., NaK tartrate) buffer; the preferred tartrate concentration is greater than 0.05 and less than about 0.2M; the most desirable value being about 0.1M. The use of phthalate, as suggested by Rhodes, U.S. 4,424,200 and 5,078,985, is unnecessary. The high tartrate concentration stabilizes the Sn(II) ions and retards the oxidation to the Sn(IV) state. As a result, precipitation of

Sn(II) or colloidal formation during buffer preparation is not usually observed. The pH of the buffer may be 4-8; a pH which results in excessive precipitation or cloudiness of the buffer, or which results in degradation and loss of immunoreactivity on the part of the antibody, should be avoided. One of the advantages of the present system is, however, the broad pH range it accommodates, allowing selection of a pH to which the antibody is insensitive. Degassing of the buffer is not essential. The pretreatment buffer is compatible with MAb stored in either normal saline or phosphate-buffered saline (PBS), and therefore the researcher may select whichever storage buffer provides better stability for the MAb.

The incubation is preferably from 8-24 hours and the incubation temperature is preferably in the range of 18-40 deg. C., and most desirably is 37 deg. C.

After this treatment, the reduced antibody may be frozen or lyophilized for storage purposes. When desired, the reduced antibody preparation may be reacted with a pertechnetate salt, e.g., Na salt, for labeling purposes. Radiolabeling efficiencies of over 90% are routinely observed, and the immunoreactivity of the antibody is essentially unaffected.

The antibody may also be incorporated into a conjugate having desirable properties. An example of such a conjugate is an immunotoxin, wherein one moiety is an antibody and another is a toxin. The antibody may target, e.g., a virus-infected cell, and the toxin then kills the cell. Useful toxins include, e.g., ricin and abrin.

The antibody may be directed against any antigen of clinical significance, but preferably is directed against a tumor-, pathogen- or parasite-associated antigen. In the case of a tumor-associated antigen (TAA), the cancer may be of the lung, colon, rectum, breast, ovary, prostate gland, head, neck, bone, immune system, or any other anatomical location. The subject may be a human or animal subject. The antibody may be a polyclonal antibody or a monoclonal antibody. When the subject is a human subject, the antibody may be obtained by immunizing any animal capable of mounting a usable immune response to the antigen. The animal may be a mouse, rat, goat, sheep, rabbit or

other suitable experimental animal. The antigen may be presented in the form of a naturally occurring immunogen, or a synthetic immunogenic conjugate of a hapten and an immunogenic carrier. In the case of a monoclonal antibody, antibody producing cells of the immunized animal may be fused with "immortal" or "immortalized" human or animal cells to obtain a hybridoma which produces the antibody. If desired, the genes encoding one or more of the immunoglobulin chains may be cloned so that the antibody may be produced in different host cells, and if desired, the genes may be mutated so as to alter the sequence and hence the immunological characteristics of the antibody produced.

The antibody may be administered to the patient by any immunologically suitable route, such as intravenous, intraperitoneal, subcutaneous, intramuscular or intralymphatic routes, however the intravenous route is preferred. The clinician may compare the anti-idiotypic and anti-isotypic responses associated with these different routes in determining the most effective route of administration.

Example I

Reduction of Antibody

Stannous ion is a known sulphydryl reductant. We use a stabilized stannous ion solution prepared from stannous chloride and tartrate salt. Controlled reduction with stannous ion of a monoclonal antibody produced a modified MAb preparation containing an average of approximately one sulphydryl group per molecule. Further evidence of sulphydryl creation is the ability of the molecule to radiolabel with Tc-99m in the presence of Tc-99m[(III),(IV)m(V)] complexes, known to form stable bonds with thiol groups. This mild controlled process does not lead to any significant loss of antigen binding properties of the MAb.

A solution containing 2.822 g of Sodium Potassium Tartrate is prepared in 98 ml of sterile water for injection and degassed of dissolved oxygen by bubbling nitrogen gas (5-10 psi) through the solution for 30 minutes. A second solution is prepared containing 1.13 g of stannous chloride in 10.0 ml of 1.0 N HCl. A quantity of 400 μl of this solution is added to the tartrate buffer solution and the mixture adjusted to pH=5.6±0.05 as

measured by a calibrated pH meter by slow addition of 1.0 N NaOH. A quantity of 40 ml of this tartrate stabilized stannous ion solution is added to 60 ml of a 5.0 mg/ml solution of MAb-170 or MAb-B43 (contained in a pH 7.4 NaH_2PO_4 buffered matrix).

MAb-170 (more accurately, MAb170H.82) is a murine monoclonal antibody of the IgG1 kappa isotype that was produced by immunizing BALB/c mice with a synthetic glycoconjugate consisting of a Thomsen-Friedenreich (TF) beta (Galbeta1->3GalNAc) disaccharide hapten coupled to an immunologically suitable carrier (serum albumin). It was selected based on its reactivity with human adenocarcinoma tissue in vitro. It clearly reacts with adenocarcinomata of the breast, ovary, endometrium, colon, prostate and some bladder. It also reacts with adenosquamous, small cell and squamous cell lung carcinoma tissue. It is described in more detail in copending Ser. No. 07/153,162, filed May 12, 1988, incorporated by reference herein, which is a continuation of Ser. No. 06/927,277, filed Oct. 27, 1986. MAb-170 has been formulated into a Tc-99m radiolabeled antibody kit (TRUSCINT AD, Biomira, Inc., Edmonton, Alberta, Canada) for radioimmunodiagnosis of adenocarcinomas. See McEwan, et al., Nuclear Medicine Communications, 13: 11-19 (1992). A hybridoma (170H82. R1808) secreting MAb 170 was deposited on July 16, 1991 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 USA, an International Depository Authority under the Budapest Treaty, and assigned the accession number HB 10825. This deposit should not be construed as a license to make, use or sell the hybridoma or MAb 170.

MAb-B43 (more accurately, B43.13) is a murine monoclonal antibody of the IgG1 kappa isotype that was produced by immunizing mice with the CA125 antigen. It was selected for its reactivity to CA 125, an ovarian carcinoma-associated antigen. It inhibits the binding of MAb OC125 to CA125. MAb B43 is reactive with CA125 antigen in biopsy tissue and in serous and endometroid carcinomas of the ovary. It has been formulated into a Tc99m-radiolabeled antibody kit (TRUSCINT OV, Biomira, Inc. Edmonton, Alberta, Canada) for radioimmunodiagnosis of ovarian carcinomas. See Capstick, et al., Int. J. Biol. Markers, 6: 129-135 (1991).

Reference to these two antibodies should not be construed as a limitation on the generality of the present invention.

The headspace of the reaction vessel containing this combination is purged with nitrogen gas and allowed to incubate for about 24 hours. Then, 0.67 ml aliquots of the solution are filtered into 5 ml nitrogen purged sterile vials and frozen at -20°C. Each vial contains nominally 2.0 mg of treated MAb-170 or MAb-B43. The final preparation is sterile, pyrogen-free and suitable for human injection.

Example II

Human Anti-Mouse Antibody (HAMA) Assays

The Biomira TRUQUANT HAMA-RIA kit (Biomira, Inc., Edomonton, ALberta, Canda) is an in vitro test for the detection of anti-idiotypic and anti-isotypic human anti-mouse antibodies (HAMA) of either the IgG or IgM subclasses, in human serum. However, the principles of the kit are more broadly applicable to the detection of anti-idiotypic and anti-isotypic antibodies.

The Biomira kit utilizes goat anti-mouse capture reagent on 1/4" polystyrene beads. Of course, other anti-mouse capture reagents could be subtituted for the goat anti-mouse antibody. This allows for capture of (a) idiotype and isotype matched or (b) idiotype mismatched, isotype matched control MAbs. Patient samples are then tested against beads that have been primed with matched and mismatched mouse antibodies. By subtracting the anti-isotype (control) response from the anti-idiotype (or matched) response, the two types of HAMA responses can be determined. Formulae for the calculation of the Total, Control, and Idiotype HAMA Indexes appear below:

Total HAMA Index (calculated using the specific or matched antibody)=CPM Sample on idiotype-specific Ab / HAMA Limit*

Control HAMA Index (calculated using the mismatched antibody)=CPM Sample on idiotype mismatched, isotype-matched Ab / HAMA Limit*

Idiotype Index = Total HAMA Index (specific) - Control HAMA Index (mismatched)

*The HAMA Limit [(0.2 x CPM of the Positive Control) + CPM of the Negative Control] used in the HAMA kit was determined to be the upper limit of normal distribution of samples from patients not injected with mouse antibodies. This run specific cutoff value establishes a level above which a >95% confidence can be used to determine that the result obtained is a true anti-mouse antibody response. The evaluation of the MAb-170 patients was based on a change of the HAMA Index from pre-injection to post injection samples. A significant change is a difference greater than 1 HAMA Index value.

Example III

Anti-Idiotype Serum Assays

The present example shows a reduced antibody elicited almost no anti-isotype response relative to an unreduced antibody. While the reduced antibody also exhibited some reduction of the anti-idiotype response, possibly as a result of cleavage of disulfide bridges near the antigen-binding site, this latter response was still substantial. MAb-170, as described above, was labeled with either Tc-99m or In-111. Labeling with Tc-99m was accomplished by first reducing the antibody as described in Example I and then reacting it with sodium pertechnetate as previously described. Labeling with In-111, to act as a control for the reduced MAb 170, did not involve any reductive process. Instead, MAb 170 was reacted with DTPA anhydride to produce a chelate attachment site for In-111 labeling. The HAMA response to a single 4-8 mg dose was determined.

The results are shown in Table 1 below.

While the HAMA kit used to measure the HAMA response used bead-bound MAb 170 in unreduced form as the capture reagent for anti-idiotype antibodies, substitution of bead-bound reduced MAb 170 did not lead to a significant change in the results obtained.

The HAMA response may also be quantified in terms of the

number of patients seroconverting to production of anti-idiotype or anti-isotype following injection of the antibody. The results are shown in Table 2 below.

Example IV

Correlation of HAMA Idiotype with Cancer Survival

In Table III, ten ovarian cancer patients injected with MAbs (fragment MAb OC 125 and reduced but unfragmented MAb B43) had a mean survival time as of the date of compilation of about three years. Of the ten patients, nine were still alive. Of these nine, two have progressing disease and 7 are stable or free of the disease. This is beyond normal expectations for these patients and is attributed to the presence of anti-idotype MAbs against the injected MAbs.

OC-125 is a murine antibody generated by the immunization of BALB/c mice with a human serous papillary cystadenocarcinoma. OC125 reacts with the CA125 antigen, which has been identified as a high molecular weight glycoprotein found on the cell surface of many ovarian cancers.

For molecular biology and immunology procedures not described above, see Sambrook, et al., Molecular Cloning: A Laboratory Manual (2nd ed., Cold Spring Harbor: 1989); Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor: 1988); Ausubel, et al., Current Protocols in Molecular Biology (Wiley Interscience: 1987, 1991).

Example V

Hama Analysis Post MAb 170 and MAb 174 Immunoscintigraphy

In support of previous findings the nonspecific and anti-isotype HAMA seroconversion rates after a single immunoscintigraphy with the reduced antibodies of the present invention is significantly lower than historical results with other antibodies/conjugates. Using the TRUQUANT HAMA RIA to measure the response to a single 1 mg dose, and comparing pre-infusion to post infusion samples, 0/22 patients developed a generalized or non-specific HAMA. Amongst patients infused with partially reduced MAb 170 (n=16), no patients showed anti-isotype or generalized HAMA responses and 2/16 seroconverted in an

idiotype specific manner. Amongst patients infused with partially reduced MAb 174 (n=6) no patients showed generalized HAMA while 1/6 did seroconvert in an idiotype specific manner. While the idiotypic-specific HAMA was less pronounced than for Example I, this may well be attributable to the lower dosage employed. In any event, the isotypic HAMA response was eliminated, while at least some idiotypic HAMA response was retained.

Table 1: HAMA response after 1 injection of either Tc-99m MAb-170 or In-111 MAb-170

Injected MAb[C] Group (n=12)	1 Month Post Inj.[A] Isotype[B]	Idiotype[B]	Idiotype	Peak Response[A] Isotype[B]	Idiotype[B]	%Idiotype
MAb-170[D] (Tc-99m labeled; reduced)	-0.07	1.11	106.7	0.1	1.61	94.2
MAb-170 (In-111 labeled; non-reduced control)	0.36	1.79	83.3	1.86	2.19	54.1

A Data was collected from pre-injection samples, 1 month post injection and 2 - 8 months post injection. Data is shown for 1 month post injection and the corresponding sample that showed the peak HAMA response (selected from the 2 - 8 month post injection samples). Mean data was generated from 12 patients in each group.

B Data is expressed in Index Units and has had the pre-injection sample data subtracted prior to reporting.

C The antibody injected was either Tc-99m MAb-170 or In-111 MAb-170 (reduced versus non-reduced antibody).

D A similar anti-isotypic response was obtained with a second control, reduced MAb B43.

Table 2: Number of Patients Seroconverting from pre-injection to post-injection (measured at peak response)

Injected Mab	# Producing Anti-isotype	# Producing Anti-idiotype
Tc-99m MAb-170 (reduced)	0/12	6/12
In-111 MAb-170 (control; non-reduced)	3/12	8/12

Note: The total number of subjects producing HAMA in the Tc-99m MAb group was 6. The total number of subjects producing HAMA in the In-111 MAb group was 8. Of these 8, 3 also produced an anti-isotype response. The point to note is that this response is typical for a first injection of MAb whereas the absence of an anti-isotype response with the Tc-99m MAb is novel since our MAb has not been fragmented and should theoretically produce the same pattern of HAMA.

Table 3: HAMA status and Survival times of ovarian cancer patients injected with OC-125 and B43.13

Patent #	Stage of Cancer	Number of MAb Injections[A]	HAMA Idiotype Positive	Survival Time (Months)[B]
1	IV	2	YES	43+
2*	III	2	YES	27+
3	I/II	2	YES	41+
4*	I/II	5	YES	27+
5	I/II	2	YES	44+
6	III	2	YES	45+
7	IV	2	YES	14
8	I/II	2	YES	52+
9*	III	2	YES	16+
10*	III	2	YES	35+

A All patients were injected with 1 mg of MAb OC 125 F(ab')2 per dose. Patients marked with a * also received 2 mg of MAb B43, reduced, unfragmented antibody.

B Patients are listed with a + if they are ongoing in the study. Patients listed without a + are deceased.

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CLAIMS

1. Use of an antibody in at least partially reduced form in the manufacture of a composition for the treatment of a disease associated with an antigen specifically bound by said antibody, said reduced antibody eliciting at least some anti-idiotypic anti-antibody response in a subject having said disease, but no more than a substantially decreased, if any, anti-isotype anti-antibody response, relative to the response which said antibody would have elicited had it been adaministered without said reduction.

2. The use of claim 1 wherein the antibody is repeatedly administered to the subject.

3. The use of claim 1 wherein the disease is a cancer and the antibody recognizes a tumor-associated antigen.

4. The use of claim 3 in which the cancer is an ovarian cancer.

5. The use of claim 3 in which the cancer is an adenocarcinoma.

6. The use of claim 1 in which the antibody is reduced with an agent selected from the group consisting of formamidine sulfonic acid, mercurous ion, stannous ion, cyanide ion, sodium cyanoborohydride, sodium borohydride, dithiothreitol, mercaptoethanol, mercaptoethanolamine, and thioredoxin.

7. The use of claim 1 in which the antibody is reduced with stannous ion.

8. The use of claim 6 in which the reduced antibody is labeled with technetium or rhenium.

9. A method of partially reducing an antibody which comprises reacting the antibody with a source of stannous ion in a tartrate buffer containing greater than 0.05M tartrate.

10. A method of radiolabeling an antibody which comprises partially reducing the antibody by the method of claim 9 to obtain an antibody with at least one free sulfhydryl group, and then reacting the partially reduced antibody with a pertechnetate or perrhenate salt to obtain a technetium- or rhenium-labeled antibody.

11. Use of a radiolabeled antibody in at least partially reduced form in the manufacture of a composition for the immunodetection by in vivo imaging of a disease associated with an antigen specifically bound by said antibody, said reduced antibody eliciting at least some anti-idiotypic anti-antibody response in a subject having said disease, but no more than a substantially decreased, if any, anti-isotype anti-antibody response, relative to the response which said antibody would have elicited had it been administered without said reduction.

INTERNATIONAL SEARCH REPORT

International Application No PCT/CA 93/00110

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 39/395,A 61 K 43/00,C 07 K 15/28,C 12 P 21/08, C 12 N 15/13,A 61 K 49/02

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 61 K,C 07 K,C 12 P,C 12 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	NUCLEAR MEDICINE COMMUNICATIONS, no. 13, 1992 (Chapman and Hall, London); A.J.B. MCEWAN et al. "MAb 170H.82: an evaluation of a novel panadenocarcinoma monoclonal antibody labelled with 99Tcm and with 111In" pages 11-19; totality. --	1,3,5, 8,11
A	US, A, 4 816 249 (LEVY et al.) 28 March 1989 (28.03.89), abstract; claims. --	1,3
A	US, A, 4 661 586 (LEVY et al.) 28 April 1987 (28.04.87), abstract; claims. ----	1,3

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
22 June 1993	06. 08. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	SCHNASS e.h.

ANHANG	ANNEX	ANNEXE
zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.	to the International Search Report to the International Patent Application No.	au rapport de recherche international relatif à la demande de brevet international n°

PCT/CA 93/00110 SAE 72361

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.	This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The Office is in no way liable for these particulars which are given merely for the purpose of information.	La présente annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche international visée ci-dessus. Les renseignements fournis sont donnés à titre indicatif et n'engagent pas la responsibilité de l'Office.

Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
US A 4816249	28-03-89	US A 4661586	28-04-87
US A 4661586	28-04-87	US A 4816249	28-03-89

PATENT COOPERATION TREATY

Amend Prior to Pub: 4/25/00

From the INTERNATIONAL SEARCHING AUTHORITY

To:
ADRIANE M. ANTLER
PENNIE & EDMONDS LLP
1155 AVENUE OF THE AMERICAS
NEW YORK, NY 10036

FORWARDED TO S. Cheng
REC'D
FEB 29 2000
Pennie & Edmonds
O.K. for filing

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference 6750-018-228	Date of Mailing (*day/month/year*) 25 FEB 2000 FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/US99/26671	International filing date (*day/month/year*) 12 November 1999 (12.11.1999)
Applicant EURO-CELTIQUE, S.A.	

1. ☒ The applicant is hereby notified that the international search report has been established and is transmitted herewith.
Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompany sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application , or of the priority claim, must reach the International Bureau as provided in rules 90 *bis* 1 and 90 *bis* 3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Ulrike Winkler, Ph.D. Telephone No. 703-308-8294

Form PCT/ISA/220 (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/26671

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 16/00
US CL : 530/387.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/131.1, 133.1, 134.1; 530/350, 387.1, 387.2, 388.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Medline, West

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	US 5,637,300 (DUNBAR et al.) 10 June 1997 (10.06.1997), column 10-11	1,4,5,10,21,22,25,26, 31 --- 11,14,15,20
Y	SEFERIAN et al. Antibody synthesis induced by endogenous internal immages. Applied Biochemisrty and Biotechnology 1994, Vol. 47, see pages 213-227.	1, 7-11, 17-21, 28-31
Y	CARRON et al. Characterization of antibodies to idiotypic determinants of monoclonal anti-sperm antibodies. Biology of Reproduction 1988, Vol. 38, see abstract.	1,2,5,10-12,15, 20-23, 26,31
Y	TRIPATHI et al. Antigen mimicry by an anti-idiotypic antibody single chain variable fragment. Molecular Immunology 1998, Vol. 35, see pages 853-863.	1,7,8-11,17-22,28-31
Y	US 5,208,146A (IRIE) 04 May 1993 (04.05.1993) see column 1 and 5.	1,7-11, 17-21, 28-31
Y	US 5,436,157A (HERR et al.) 25 July 1995 (25.07.1995) see columns 1-3.	2,3,6,12,13,16,23,24, 27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 January 2000 (28.01.2000)	25FEB2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Ulrike Winkler, Ph.D. Telephone No. 703-308-8294

Form PCT/ISA/210 (second sheet) (July 1998)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 6750-018-228	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US99/26671	International filing date (*day/month/year*) 12 November 1999 (12.11.1999)	(Earliest) Priority Date (*day/month/year*) 13 November 1998 (13.11.1998)
Applicant EURO-CELTIQUE, S.A.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the Report**
   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
   ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:
   ☐ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readable form.
   ☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.
2. ☐ **Certain claims were found unsearchable** (See Box I).
3. ☐ **Unity of invention is lacking** (See Box II).
4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:
5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.
6. The figure of the **drawings** to be published with the abstract is Figure No.
   ☐ as suggested by the applicant. ☒ None of the figures
   ☐ because the applicant failed to suggest a figure.
   ☐ because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/26671

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C07K 16/00
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Medline, West

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Y	CARRON et al. Characterization of antibodies to idiotypic determinants of monoclonal anti-sperm antibodies. Biology of Reproduction 1988, Vol. 38, see abstract.	1,2,5,10-12,15, 20-23, 26,31
Y	TRIPATHI et al. Antigen mimicry by an anti-idiotypic antibody single chain variable fragment. Molecular Immunology 1998, Vol. 35, see pages 853-863.	1,7,8-11,17-22,28-31
Y	US 5,208,146A (IRIE) 04 May 1993 (04.05.1993) see column 1 and 5.	1,7-11, 17-21, 28-31
Y	US 5,436,157A (HERR et al.) 25 July 1995 (25.07.1995) see columns 1-3.	2,3,6,12,13,16,23,24, 27

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 January 2000 (28.01.2000)	25 FEB 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Ulrike Winkler, Ph.D. Telephone No. 703-308-8294

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